PB86-118270

Drinking Water Criteria Document for Viruses (Final Draft)

(U.S.) Environmental Protection Agency
Cincinnati, OH

Jun 85

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

FINAL DRAFT
ECAO-CIN-451
June, 1985

EPA

Research and Development

PB86-118270

DRINKING WATER CRITERIA DOCUMENT FOR VIRUSES

Prepared for

OFFICE OF DRINKING WATER

Prepared by

Environmental Criteria and
Assessment Office
Cincinnati OH 45268

DRAFT: DO NOT CITE OR QUOTE

NOTICE

This document is a preliminary draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comments on its technical accuracy and policy implications.

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

2.

3. RECIPIENT'S ACCESSION NO.
PB86 118270 /AS

SUBTITLE
Drinking Water Criteria Document for Viruses

5. REPORT DATE
4/84.

6. PERFORMING ORGANIZATION CODE

HOR(S)

8. PERFORMING ORGANIZATION REPORT NO.

PERFORMING ORGANIZATION NAME AND ADDRESS
Same as box 12.

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Office of Drinking Water (WH-550)
401 M St., S.W., Washington, D.C. 20460

13. TYPE OF REPORT AND PERIOD COVERED
Scientific review

14. SPONSORING AGENCY CODE
EPA/CSD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The Office of Drinking Water, U.S. Environmental Protection Agency, has prepared a drinking water criteria document on viruses. This document includes an extensive review of the following topics:

- Occurrence in water
- Health effects in animals
- Health effects in humans
- Mechanisms of disease
- Risk assessment

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Viruses Drinking Water		

18. DISTRIBUTION STATEMENT
OPEN

19. SECURITY CLASS *(This Report)*
Non-sensitive

20. SECURITY CLASS *(This page)*
Non-sensitive

21. NO. OF PAGES
240

22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

DISCLAIMER

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

AVAILABILITY NOTICE

This draft document is for internal review and should not be released. For information contact Dr. Jerry F. Stara, Director, Environmental Criteria and Assessment Office, Cincinnati, OH (513/684-7531).

DOCUMENT DEVELOPMENT

Jerry F. Stara, D.V.M., Co-Document Manager
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Cynthia Sonich Mullin, M.S., Co-Document Manager
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Vlašta Molak, Ph.D., Co-Document Manager
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Helen H. Ball, Project Officer
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Authors

Gerald Berg, Ph.D.
Director of Research
The ROMIST Corporation
and
Research Professor
Dept. of Civil and Environmental Engineering
University of Cincinnati

Scientific Reviewers

Elmer W. Akin, Ph.D.
Toxicology and Microbiology Division
Health Effects Research Laboratory, Cincinnati
U.S. Environmental Protection Agency

Paul Berger, Ph.D.
Office of Drinking WAter
U.S. Environmental Protection Agency
Washington, DC

Charles P. Gerba, Ph.D.
Dept. of Microbiology and Immunology
The University of Arizona
Tucson, Arizona

Mark D. Sobsey, Ph.D.
The School of Public Health
Dept. of Environmental Sciences and Engineering
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

TABLE OF CONTENTS

Page

I. SUMMARY I-1

II. GENERAL INFORMATION AND PROPERTIES. II-1

INTRODUCTION. II-1
THE VIRUSES IN WATER. II-2

Sources of the Viruses in Water. II-2
Physical Description of the Viruses in Water II-4

EPIDEMIOLOGY. II-11

Epidemiological Evidence for Waterborne Transmission of Viruses. II-11
Seasonal Distribution of Viruses in Water. II-13
Waterborne Outbreaks of Viral Diseases in the United States. II-13

SUMMARY II-24

III. OCCURRENCE IN WATER III-1

VIRUSES IN ENVIRONMENTAL WATERS III-1

Viruses of Man III-1
Viruses of Animals III-1
Viruses of Fish. III-2
Viruses of Plants. III-2
Bacterial Viruses. III-3

VIRUSES IN SEWAGE III-3

Factors That Affect the Numbers of Viruses in Sewage . . III-5
Recovery of Viruses from Sewage. III-8

VIRUSES IN SURFACE WATERS III-17

Survival of Viruses in Surface Waters. III-17
Physical Factors III-22
Chemical Factors III-22
Biological Factors III-23
Recovery of Viruses from Surface Waters. III-24

VIRUSES IN GROUNDWATERS III-28
VIRUSES IN DRINKING WATERS. III-31
SUMMARY III-34

TABLE OF CONTENTS (cont.)

Page

IV. HEALTH EFFECTS IN ANIMALS IV-1

DISEASES OF ANIMALS PRODUCED BY ENTERIC VIRUSES OF HUMANS . . IV-1

Enteroviruses. IV-1
Other Viral Genera IV-6

MINIMAL INFECTIVE DOSE OF HUMAN VIRUSES IN ANIMAL HOSTS . . . IV-9

V. HEALTH EFFECTS IN HUMANS. V-1

DISEASES OF MAN PRODUCED BY ENTERIC VIRUSES OF MAN. V-1

Enteroviruses. V-1
Other Viral Genera V-12

MINIMAL INFECTIVE DOSE OF HUMAN VIRUSES IN HUMANS V-20

VI. MECHANISMS OF DISEASE VI-1

FACTORS THAT AFFECT DISEASE OCCURRENCE. VI-1

Virulence. VI-1
The Susceptibility of Host Cells VI-1

STATES OF DISEASE VI-2

Apparent Infections. VI-2
Inapparent Infections. VI-3

HOST DEFENSE SYSTEMS. VI-4

Antibodies VI-4
Cell-Mediated Immunity VI-5
Nonspecific Factors in Immunity. VI-6

VII. RISK ASSESSMENT VII-1

INTRODUCTION. VII-1
TRANSMISSION OF VIRUSES BY DRINKING WATER VII-2

Reasons for Continuing Concern VII-2
Epidemiological Evidence VII-2
Viruses at Water Intakes and in Drinking Waters. VII-9
Minimal Infective Dose VII-11

TABLE OF CONTENTS (cont.)

Page

THE NEED FOR STANDARDS. VII-12

Magnitude of the Problem VII-13
Mode of Transfer of Enteric Infections VII-13
Personal Contact Transmission from Community to Community. VII-14
The Water Transmission Scenario. VII-14
Other Considerations VII-15

RECOMMENDED STANDARDS FOR VIRUSES IN WATERS (non-U.S. EPA). . VII-17

Drinking Waters (non-U.S. EPA) VII-17
Renovated Waters (non-U.S. EPA). VII-18

THE BACTERIOLOGICAL STANDARD FOR DRINKING WATERS. VII-19
APPRAISAL OF RISK VII-21
SUMMARY VII-22

VIII. METHODOLOGY VIII-1

INTRODUCTION. VIII-1
METHODS FOR CONCENTRATING AND RECOVERING VIRUSES FROM DRINKING WATER. VIII-1

Negatively Charged Virus-Adsorbing Filters VIII-3
Positively Charged Virus-Adsorbing Filters VIII-5

DEFICIENCIES OF CURRENT VIRUS RECOVERY METHODOLOGY. VIII-5
METHODS FOR DETECTING VIRUSES IN DRINKING WATER VIII-7

IX. POTENTIAL INDICATORS FOR WATERBORNE VIRUSES IX-1

THE IDEAL INDICATOR OF VIRUSES IN DRINKING WATER. IX-1
LOGIC IN CHOICE OF INDICATOR SYSTEMS. IX-1
TOTAL COLIFORMS IX-2
ALTERNATIVE INDICATOR SYSTEMS IX-3

Fecal Coliforms. IX-4
Vegetative Bacteria (Total counts) IX-5
Fecal Streptococci IX-6
Clostridium perfringens. IX-8
Bifidobacteria IX-9
Mycobacteria and Yeasts. IX-9
Bacterial Viruses (Bacteriophages) IX-10

CONCLUSIONS IX-14

X. REFERENCES. X-1

LIST OF TABLES

No.	Title	Page
II-1	Human Enteric Viruses that may Occur in Water	II-3
II-2	Waterborne Outbreaks of Viral Etiology in the United States	II-15
II-3	Drinking Water and Recreational Water Outbreaks of Norwalk Virus-Induced Disease in the United States	II-16
II-4	Drinking Water Outbreaks of Rotavirus-Induced Disease	II-18
II-5	Waterborne Outbreaks of Infectious Hepatitis (probably hepatitis A) in the United States, 1946-1982	II-20
II-6	Waterborne Outbreaks of Norwalk Virus-Induced Disease	II-21
III-1	Viruses in Sewage in an Arid Land	III-9
III-2	Viruses in Primary Sewage Effluents	III-11
III-3	Distribution of Enteroviruses in Activated Sludge Aeration Basins	III-12
III-4	Removal of Viruses from Raw Sewage by an Oxidation Ditch and by a Trickling Filter	III-13
III-5	Adsorption of Poliovirus 1 to Activated Sludge Solids	III-19
III-6	Recovery of Viruses from Bay Waters and Sediments	III-20
III-7	Survival of Seeded Poliovirus 1 in Freshwater and in Seawater	III-25
III-8	Recoveries of Viruses from Surface Waters	III-26
III-9	Recoveries of Viruses Along the Mississippi and Missouri Rivers	III-27
III-10	Numbers of Enteric Viruses in Samples of River Waters Collected at Less Than 2-Minute Intervals	III-29
III-11	Reported Recoveries of Human Viruses from Treated Drinking Waters	III-32

LIST OF TABLES (cont.)

No.	Title	Page
IV-1	Infective Dose by the Respiratory Route of Coxsackievirus B4 for Newborn Mice.	IV-10
IV-2	Infective Dose by the Intranasal Route of Myxovirus parainfluenza 3 for Hamsters.	IV-11
IV-3	Infective Dose by Inhalation of Rift Valley Fever Virus for Hamsters.	IV-12
V-1	Association of Clinical Syndromes with Group A Coxsackieviruses.	V-5
V-2	Association of Clinical Syndromes with Group B Coxsackieviruses.	V-7
V-3	Associations of Clinical Syndromes with Echoviruses	V-9
V-4	Clinical Syndromes Produced by Adenoviruses	V-14
V-5	Infections of Humans with the Attenuated SM Strain of Poliovirus 1	V-23
V-6	Infection of Infants with an Attenuated Strain of Poliovirus 3.	V-24
V-7	Infection of Infants with an Orally Administered Attenuated Strain of Poliovirus 1	V-25
V-8	Infection of Children Intranasally with Attenuated Measles Vaccine	V-26
V-9	Infection of Healthy Adults with Echovirus 12	V-27
VII-1	Waterborne Outbreaks of Hepatitis A in the United States from 1973-1982	VII-4
VII-2	Waterborne Outbreaks of Norwalk and Norwalk-like Virus Diarrhea in the United States from 1978-1982.	VII-5
VII-3	Water Systems Involved in Outbreaks of Viral Disease in the United States, 1973-1982	VII-6
VII-4	Limits for Enteric Viruses and Fecal Coliforms in Effluent Dominated Waters in the State of Arizona	VII-20
VII-5	Virus Concentrations in Water and Expected Illness.	VII-23

LIST OF TABLES (cont.)

No.	Title	Page
VIII-1	Methods for Concentrating Viruses from Water.	VIII-2
VIII-2	Commonly Used Cell Cultures for Propagating Human Enteric Viruses	VIII-8
IX-1	Persistence of Indicator Organisms and Pathogenic Bacteria in Municipal Drinking Water at 20°C.	IX-7

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
BGM	Buffalo green monkey
CFU	Colony-forming unit
CNS	Central nervous system
CPE	Cytopathic effect
DNA	Dioxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
GI	Gastrointestinal
HFDK	Human fetal diploid kidney
HID$_1$	1% human infective dose
HID$_{50}$	50% human infective dose
HT	High temperature
IAHA	Immune adherence hemagglutination assay
ID$_{50}$	50% infective dose
IPN	Infectious pancreatic necrosis
IgA	Immunoglobulin A
IgG	Immunoglobulin G
LD$_{50}$	Dose lethal to 50% of recipients
LT	Low temperature
MAVA	Marine antiviral agent
MPNCU	Most probable number of cytopathic units
NTU (IS)	Nephelometric turbidity units
PFU	Placque-forming unit
RD	Rhabdosarcoma

LIST OF ABBREVIATIONS (cont.)

RIA	Radioimmunoassay
RNA	Ribonucleic acid
$TCID_{50}$	50% tissue culture infective dose

I. SUMMARY

More than 120 different viruses that infect and multiply in or around the GI tract of man have been classified. These viruses are excreted by infected individuals into domestic sewage that is usually discharged, with or without treatment, to rivers or streams impacting downstream recreational waters, water intakes, and perhaps shellfish beds in estuaries. When sewage is discharged to the land, it may impact groundwaters. Viruses are readily recovered from rivers miles downstream of outfalls and at water intakes, and from groundwaters impacted by discharges to the land.

Viruses that multiply in or near the GI tract of man are known as the enteric viruses, consisting of the enteroviruses (polioviruses, groups A and B coxsackieviruses, echoviruses, and other enteroviruses including hepatitis A virus), reoviruses, rotaviruses, adenoviruses, Norwalk and Norwalk-like viruses, and a number of other small round viruses. Many of these viruses are responsible for a large variety of human illnesses including poliomyelitis, aseptic meningitis, infectious hepatitis, herpangina, pleurodynia, gastroenteritis and rashes.

For many decades, except for one outbreak of poliomyelitis, only infectious hepatitis had been proven to be waterborne. In recent years, improved technology has resulted in the identification of at least six waterborne outbreaks of rotavirus gastroenteritis, 17 waterborne outbreaks of gastroenteritis produced by Norwalk virus, and several other waterborne outbreaks produced by Norwalk-like viruses. A large proportion of the nonbacterial gastroenteritis epidemics of earlier years were likely caused by these agents. Moreover, because of the lack of routine surveillance systems necessary to identify disease outbreaks, and especially waterborne outbreaks, most epidemics are probably never detected.

Although many outbreaks of waterborne disease are small, they can be large and devastating. An outbreak of infectious hepatitis in New Delhi, India in 1955 and 1956 produced tens of thousands of illnesses and many deaths. A waterborne outbreak of gastroenteritis in Georgetown, Texas in 1980 produced ~8000 cases out of a population of ~10,000. The gastroenteritis epidemic was followed by an associated outbreak of hepatitis A that produced 36 illnesses.

All enteric viruses occur in sewage, although not all are readily detected because the methods used for recovering viruses were designed primarily for enteroviruses. In most studies, only one cell culture system sensitive to many enteroviruses is used to detect the viruses recovered from environmental waters with concentration techniques. Many other enteroviruses (including most group A coxsackieviruses) do not grow in these cell culture systems. Adenoviruses, rotaviruses, hepatitis A virus, Norwalk, Norwalk-like and other small round viruses grow poorly or not at all in many cell culture systems. Thus, the viruses that are known to produce waterborne epidemics (hepatitis A virus, Norwalk virus, Norwalk-like viruses, and the rotaviruses) are not detected in such studies. Detection of these viruses requires newly developed, specialized and relatively difficult techniques.

Wastewaters in the United States usually contain between 25 and 250 enteroviruses per liter. In warm climates with poor sanitary conditions or limited availability of water, enteroviruses $\geq$250,000/ℓ of sewage have been reported. The viruses in sewage reflect the presence of viruses in the community that produces the sewage. Although the polioviruses, because of vaccination programs, may be found in sewage in large numbers throughout the year, most enteroviruses occur primarily during the warmer months. Hepatitis A virus, rotaviruses, and some adenoviruses exist in the community and presumably in its sewage in the colder months. Viruses in environmental waters other than sewage reflect these patterns. Norwalk virus and Norwalk-like virus outbreaks occur in all seasons. Because of the inefficiency, insensitivity and costs of detection methods, the number of viruses reported are considerably lower than the numbers actually present in environmental waters.

Viruses in surface waters originate from sewage outfalls upstream and from the discharges of isolated residences nearest to the surface waters; they survive for long periods in fresh waters. Many viruses are adsorbed or embedded in fecal matter or cellular detritus, or adsorbed to solids in these waters; they are to some degree protected from noxious elements in the water environment. Often, adsorbed viruses settle to the bottom of streams and are churned up by heavy rains or runoff at a later time. Many viruses reach the recreational waters and water intakes of downstream communities and often the shellfish beds in estuarine waters. Viruses are often recovered from surface waters far downstream of outfalls and at water intakes.

Groundwaters are increasingly impacted by sewages and sludges discharged to the land. Viruses have been recovered from groundwaters.

There are many reports of virus isolations from drinking water supplies that received complete treatment, including disinfection. Complete engineering data for treatment plants at which viruses were recovered are available in several instances. Viruses have been recovered from chlorinated drinking waters, negative for total coliforms, exposed to 0.5 mg/ℓ of free chlorine for at least 30 minutes and turbidities of <1 NTU.

Minimal infective dose data are available for relatively few viruses; most studies were performed with relatively safe vaccine strains. These data indicate that very few cell culture infective doses* of viruses are necessary to produce infection in man. Sometimes only one cell culture infective dose seems necessary to infect most or all inoculated individuals. Sometimes only one of several individuals inoculated with one or a few cell culture infective doses becomes infected. The closer to susceptible cells inoculation brings the virus particle, the more likely it is that infection will occur.

---

*Cell cultures are used to measure the infectivity of viruses. Ideally, the infectivity in cell cultures of the viruses in drinking water samples would equal the infectivity for the same cell cultures of strains of viruses that when tested in man produce infection when one cell culture infective dose is inoculated. Although this ideal may not often be achieved, it is probably approached, because usually the most sensitive cell lines reasonably available are used both to assay the viruses inoculated into man and to recover viruses from drinking waters.

Those favoring standards for viruses in drinking waters argue that the presence of viruses in drinking waters negative for total coliforms and meeting generally accepted turbidity and chlorination standards require direct testing for the viruses.

Those against standards argue that insufficient epidemiological evidence demonstrating widespread transmission of viruses by the water route exists and that high cost of routine tests for viruses would not be justified.

A suggestion that a coliphage indicator serve as an indicator for viruses of man is in abeyance because no single coliform host is susceptible to infection by all coliphages that may occur in drinking water. Moreover, few data correlate the presence of coliphages and the presence of viruses of humans in drinking water. Few data correlate the presence of viruses of humans with presence in drinking water of fecal streptococci, Clostridium perfringens, bifidobacteria, mycobacteria, yeasts, or other possible substitutes for viruses.

The total coliform test, almost universally used as the test for the microbiological safety of drinking water, is intended to provide a large margin of safety against the possibility that pathogenic enteric bacteria could be present in drinking waters (even though hundreds and often tens of thousands of these organisms must be ingested to produce infection). Viruses, however, can produce infection when ingested in much smaller numbers, and viruses have been detected in drinking waters that are negative for total coliforms.

In 1975, a WHO Scientific Group recommended that there should be no viruses in drinking water and, to assure that this goal is achieved, 100-1000 ℓ samples of water should be tested by the best method available. WHO expert groups still recommend that there should be no viruses in drinking water. In 1980, a Conference on Monitoring for Viruses in the Environment recommended to the U.S. EPA (which convened the conference) that there should be no human enteric viruses in drinking water; in essence they endorsed the WHO Scientific Group's recommendation. The Conference group recommended that the limit for drinking water should be less than one virus per 1000 ℓ of water.

Filter adsorption-elution methods available for concentrating viruses from waters are capable of processing more than 1000 ℓ of drinking water in the field or in the laboratory. The equipment used for this purpose is still somewhat cumbersome, but transportable. Although not highly efficient, these methods constitute the technology that has frequently detected viruses in rivers and at water intakes, and also detected viruses in finished drinking waters. Because no one system can detect all viruses, a number of different methods must be used for the detection of viruses in drinking water. Most viruses can be detected and quantified by the cytopathic effect they produce in cell cultures or by the plaque technique. Different groups of viruses, and sometimes different viruses within a group, grow best in different cells. Thus, to recover all of the viruses in a water sample that can be detected by such techniques, several different cell lines should be used. Moreover, some viruses that probably occur in water have not yet been grown in cells in culture and might be detected by immune electron microscopy or by other sophisticated methods.

II. GENERAL INFORMATION AND PROPERTIES

Introduction

More than 120 different viruses multiply in the GI tract of man and are excreted with the feces of infected individuals. These viruses become a part of the sewage into which they are discharged (Berg, 1973a,b, 1978b). Although exposure to treatment processes may remove or destroy some of them, many of the excreted viruses enter our waterways. Here, they become a potential hazard to recreationalists, a problem for downstream communities that use the waterways as raw water sources for their drinking waters, and eventually a hazard to those who consume shellfish harvested from the estuaries into which the waterways empty (Akin et al., 1971; Berg, 1978b). If sewage or effluents are discharged to the land instead, viruses may contaminate crops or groundwaters (Berg, 1978b).

Not many decades ago, viruses could be detected only occasionally in raw sewage. There appeared to be no need for concern that drinking water might be a vehicle for the transmission of virus diseases. The available technology that detected viruses in sewage seldom detected viruses in rivers, and only rarely in streams. Therefore, the possible pollution of many of the Nation's drinking water supplies with viruses was of little concern.

For decades following Melnick's recovery of polioviruses from New York City sewage during the poliomyelitis outbreaks in the early 1940s (Melnick, 1978) and the subsequent demonstration shortly thereafter by Neefe and Stokes (1945) that infectious hepatitis (probably hepatitis A) could be waterborne, methods for recovering viruses from waters were developed that frequently detected viruses even at water intakes (Berg, 1978a). Moreover, researchers have documented recoveries of viruses from reportedly well-treated drinking water supplies. The extent of the virus problem seems to increase as technology for recovering viruses from the environment improves. The viruses have always been there; our capability for detecting them has changed. Thus, concern for viruses in the water environment has grown. Despite the proven presence of viruses in drinking water, it has been difficult to demonstrate the importance of water as a vehicle in the transmission of viruses from person to person, and thereby the need for standards for viruses in drinking water.

The Viruses in Water

Sources of the Viruses in Water. Enteroviruses (polioviruses, groups A and B coxsackieviruses, echoviruses, hepatitis A virus, and several other recently classified enteroviruses), rotaviruses and other reoviruses, adenoviruses, Norwalk and Norwalk-like viruses, and some other small round viruses (more than 120 serological types in all) are the most common viruses of human origin that occur in sewage. These viruses and the diseases with which they are associated are listed in Table II-1. Man is the only known animal reservoir for all of these viruses except the reoviruses. Other viruses have been recovered from human urine and feces, but none of these viruses have been recovered very often from sewage. Sewage also contains many viruses of nonhuman origin. The consequences to man of viruses of nonhuman origin in water are not known.

TABLE II-1

Human Enteric Viruses that may Occur in Water[a]

Viruses [b]	Number of Types	Diseases Caused in Humans
Enterovirus:		
Poliovirus	3	paralysis, meningitis, fever
Echovirus	32[c]	meningitis, respiratory disease, rash, diarrhea, fever
Coxsackievirus A	23	herpangina, respiratory disease; meningitis, fever
Coxsackievirus B	6	myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia, diabetes
New Enterovirus types 68-72	5	meningitis, encephalitis, respiratory disease, acute hemorrhagic conjunctivitis, fever
Including Hepatitis A virus (enterovirus [illegible]		infectious hepatitis
Norwalk virus	1	vomiting and diarrhea, fever
Norwalk-like viruses	3	vomiting and diarrhea, fever
Rotavirus	4	vomiting and diarrhea, primarily in children
Reovirus	3	not clearly established
Adenovirus	41	respiratory diseases, eye infections, gastroenteritis
Calicivirus	1	gastroenteritis
Astrovirus	1	gastroenteritis
Other small round viruses	NR	gastroenteritis
Non-A, Non-B hepatitis virus	NR	hepatitis

[a] Source: Revised and modified from Melnick (1978) and Melnick and Gerba (1982)

[b] Other stable viruses that might contaminate water are: SV40-like papovaviruses. These viruses occur in the urine. The JC subtype is associated with progressive multifocal leukoencephalopathy. Creutzfeld-Jakob disease virus. Like scrapie virus, the C-J virus resists heat and formaldehyde. The virus causes a spongiform encephalopathy characterized by severe progressive dementia and ataxia. Adenovirus-associated satellite viruses. These viruses are excreted in stools of children. The viruses survive heating for 1 hour at 60°C.

[c] Coxsackievirus A23 is identical to echovirus 9, and the former is no longer used. Echovirus 10 has been reclassified and is now reovirus 1. Echovirus 28 has been reclassified as a rhinovirus.

NR = Not reported

Physical Description of the Viruses in Water.

Enteroviruses --

Physical Characteristics. The genus *Enterovirus*, which comprises the polioviruses (3), group A coxsackieviruses (23), group B coxsackieviruses (6), echoviruses (32), and a group of new enteroviruses (enteroviruses 68-72) that includes hepatitis A virus (5), includes many of the smallest of the viruses. The genus belongs to the family Picornaviridae (small RNA viruses). These viruses measure 27-30 nm in diameter and contain a dense core ~16 nm in diameter. The capsids are icosahedral in symmetry, and each contains 32 capsomeres. The nucleic acid of the enteroviruses is a single molecule of single-stranded RNA. The remainder of the virion consists of one minor and four major proteins. Enteroviruses do not possess envelopes and are stable over a pH range of 3-9. The enteroviruses can be readily differentiated from each other and are identified by neutralization tests and by other serological tests (Lennette and Schmidt, 1979).

Host Range. The only natural host for enteroviruses of human origin is man. Experimentally, however, the polioviruses can infect Old World monkeys and chimpanzees by the oral, intracerebral and intraspinal routes. Coxsackieviruses infect suckling mice experimentally, but different lesions are produced by groups A and B. Most of the echoviruses are not known to infect any host other than humans. However, 14 echoviruses can by intracerebral or intraspinal inoculation produce neuronal lesions and meningitis in rhesus and cynomolgus monkeys, and two of these viruses can produce lesions in newborn mice similar to those produced by group A coxsackieviruses (echovirus 9) and group B coxsackieviruses (echovirus 6).

Moreover, enterovirus 72 (hepatitis A virus) can infect chimpanzees, marmosets, and some species of monkeys experimentally, and can infect chimpanzees naturally by transmission from an infected human carrier (Lennette and Schmidt, 1979).

Most enteroviruses grow in primate cell cultures at 37°C. Some group A coxsackieviruses, however, can be recovered only in newborn mice, and enterovirus 70, the etiological agent of acute hemorrhagic conjunctivitis, must be grown at 33°C, a property of the rhinoviruses (another genus of the Picornaviridae) rather than of the enteroviruses (Lennette and Schmidt, 1979).

Adenoviruses --

Physical Characteristics. Adenoviruses belong to the family Adenoviridae. They are icosahedral in symmetry and 60-90 nm in diameter. The adenovirus capsid contains 252 capsomeres, and a fiber protrudes from each of the capsid's 12 vertices. The fiber is the point of attachment of the virion to the cell it infects. Adenovirus nucleic acid is a single molecule of double-stranded DNA. The virion has no envelope. The adenovirus contains at least 10 species of proteins in its capsid and in its DNA-protein core. All strains of human adenoviruses classified contain a common antigen and cross react by complement fixation and by other serological tests (Matthews, 1982).

Host Range. None of the adenoviruses that infect man infect any other species naturally, and most of these adenoviruses are not infective to common laboratory animals. Inapparent infections, however, have been produced by intranasal inoculation of baby pigs, hamsters, dogs and guinea pigs, and by intravenous inoculations of rabbits (Lennette and Schmidt, 1979). Some of these adenoviruses are capable of producing tumors in newborn animals but apparently not in man. Most, but not all, adenoviruses grow well in vitro in a variety of mammalian cell lines. Adenoviruses 40 and 41 (so-called enteric adenoviruses), which have been detected primarily by EM examination of stools, are fastidious but have been grown in Graham 293 cells (Takiff et al., 1981) and also in several other cell types (deJong et al., 1983).

Reoviruses and Rotaviruses --

Physical Characteristics. Reovirus and rotavirus are two of the three genera that consitute the family Reoviridae. More than 150 members of this family have been classified. These viruses infect man and other vertebrates, invertebrates and plants. Reoviruses and rotaviruses are 70-80 nm in diameter and contain double-stranded, segmented RNA genomes. Reoviruses and rotaviruses have double capsids, both of which are icosahedral in symmetry. The virions have no envelopes. The capsids each contain 8 or 10 polypeptides (Matthews, 1982).

Host Range. The natural host range for reoviruses includes man and almost all other species tested. Viruses of this genus can be recovered in many available cell lines. Rotaviruses are the major etiological agent of sporadic acute enteritis in infants and young children. The rotaviruses recovered from man and those recovered from calves, mice, monkeys and offal are similar immunologically. The human rotaviruses can infect piglets, gnotobiotic newborn calves, and newborn rhesus monkeys. Rotaviruses have been propagated in a number of mammalian cell lines, but these viruses do not grow well in those lines. The identification of rotaviruses in diarrheal disease has depended upon detection of the viruses in feces by immune EM and by ELISA. Other serological methods have not been as sensitive as ELISA. Rotaviruses can now be recovered from human stools directly in cell cultures (Wyatt et al., 1983), but only a little more than 50% of stools that contain rotaviruses by EM techniques or by ELISA yield viruses in cell cultures. A recently reported dot hybridization assay that recognizes rotavirus RNA in feces seems to be sensitive and specific (Flores et al., 1983).

Recently, rotaviruses antigenically distinct from all classified rotaviruses have been recovered from human feces (Dimitrov et al., 1983; Nicolas et al., 1983). The new agents have been termed pararotaviruses. These antigenically distinct viruses do not contain the groupspecific antigen common to all of the other rotaviruses recovered from mammals or birds. The pararotaviruses, like the rotaviruses, contain 11 double-stranded RNA segments, but pararotavirus RNA produces a unique electrophoretic migration pattern.

Non-A, Non-B Hepatitis -- In recent years, several reports have appeared in the literature suggesting that several outbreaks of waterborne infectious hepatitis in India were caused by an agent that was neither hepatitis A (enterovirus 72), hepatitis B virus, nor the non-A, non-B hepatitis agent associated with transmission through human blood or blood products (Khuroo, 1980; Wong et al., 1980; Pavri et al., 1982; Purcell, 1982). Little is known about the physical nature or host range of whatever agent may be involved, although the agent is now reported to be a 27 nm particle (Sreenivasan et al., 1984):

> The search over the years with the electron microscope for viral agents in the feces of patients with diarrhea has resulted in the visualization of many small round viruses. The first of these detected was the Norwalk virus, a 27-nm particle somewhat amorphous in appearance. Subsequently, somewhat similar particles that were immunologically distinct from Norwalk virus were detected and have been termed Norwalk-like viruses. Other small round viruses have been classified as caliciviruses, astroviruses, coronavirus-like viruses and others. These agents are described below.

Norwalk Viruses --

Physical Characteristics. Norwalk virions are about 27 nm in diameter. They are amorphous in morphology, but probably icosahedral in symmetry. The virions have no envelopes. The nature of the viral nucleic acid has not yet been determined. When that is determined, the virus will probably be classifed as a parvovirus or a calicivirus (Matthews, 1982).

Host Range. The Norwalk viruses have been detected and characterized by EM techniques and defined serologically by immune EM and RIA (Blacklow et al., 1979). They have yet to be cultivated *in vivo* outside of the human host or *in vitro*.

Norwalk-like Viruses --

Physical Characteristics. The Norwalk-like viruses, also referred to as parvovirus-like, are 25-35 nm in diameter and appear be amorphous. These viruses originate from stools collected from patients during outbreaks of gastroenteritis. The Norwalk-like viruses include the Hawaii, Snow Mountain, Marin County, W-Ditchling, Cockle and Paramatta agents. At least, Hawaii, Snow Mountain and the Marin County agents appear to be immunologically distinct from each other and from the Norwalk virus. At least four immunologically distinct Norwalk-like viruses thus appear to exist (Wyatt et al., 1984).

The Otofuke agent is a somewhat larger Norwalk-like virus recovered during several outbreaks of diarrhea in Japan (Taniguchi et al., 1981). The Otofuke agent appears to be 35-40 nm in diameter. The agent appears to be distinct from the Norwalk virus and from W. Ditchling by immune EM (Taniguchi et al., 1979).

Host Range. Same as for Norwalk viruses.

Astrovirus --

Physical Characteristics. Astroviruses are ~30 nm in diameter and derive their name from the six-pointed star appearance of the virion. These viruses have been detected by EM in the stools of children with mild gastroenteritis. Astroviruses have been recovered from the feces of patients with gastroenteritis in a number of outbreaks (Konno et al., 1982). In one study, more than 70% of the children surveyed had acquired antibodies to gastroenteritis-associated astroviruses by the age of 4 (Kurtz and Lee, 1978).

Host Range. Astroviruses have been detected in the feces of patients with gastroenteritis only by EM techniques. These viruses have not been grown *in vivo* except in human hosts. Only recently have they been propagated *in vitro* (Lee and Kurtz, 1981).

Calicivirus --

Physical Characteristics. A calicivirus derived from the stools of patients with diarrheal disease is now recognized as an etiological agent of gastroenteritis. The virus appears to be unrelated immunologically to any of the other known animal caliciviruses (Matthews, 1982).

The calicivirus derived from human stools is 31-35 nm in diameter and is characterized by cup-shaped (chalice-like) indentations demonstrable by EM examination of negatively-stained specimens (Schaffer, 1979).

Outbreaks of calicivirus gastroenteritis have been reported from several nations. Serum antibodies are acquired by many children by the age of 2 years (Sakuma et al., 1981), a characteristic shared with rotavirus infections. Symptoms are also similar for rotavirus and calicivirus infections, but calicivirus infections may be milder.

Although Norwalk virus may belong to the family Caliciviridae (Greenberg et al., 1981), the Norwalk virus and the diarrhea-related calicivirus appear to be unrelated. Moreover, the calicivirus appears to produce infections at a much younger age than Norwalk virus and has not yet been shown to be water-transmitted. Norwalk virus may be transmitted largely by the water route (Kaplan et al., 1982).

Host Range. Human caliciviruses apparently do not cross react with animal caliciviruses of nonhuman origin and have not been propagated *in vivo* in nonhuman hosts. Only recently have they been propagated *in vitro* (Cubitt and Barrett, 1984). Human caliciviruses have been demonstrated primarily by

EM techniques and characterized immunologically by immune EM. A solid-phase RIA for human caliciviruses, however, has recently been reported (Nakata et al., 1983).

Other Small Round Viruses -- A number of small round particles different from those described previously have been detected by EM in the feces of patients with gastroenteritis. None of these particles are clearly etiological agents of gastroenteritis. Included among these small round particles are a coronavirus-like particle, a so-called minireovirus and a so-called minirotavirus.

The coronavirus-like particles have been detected as frequently in the feces of healthy children and adults as in the feces of children and adults with gastroenteritis (McNaughton and Davies, 1981).

Minireoviruses and minirotaviruses are 33-40 nm particles that have been detected by EM techniques in infants with diarrhea. The precise role of these viruses in the etiology of gastroenteritis is yet to be determined (Riepenhoff-Talty et al., 1983).

Host Range. No host other than man is known.

Epidemiology

Epidemiological Evidence for Waterborne Transmission of Viruses. Although many outbreaks of waterborne infectious hepatitis (probably hepatitis A) and some outbreaks of viral gastroenteritis have been reported, there is little epidemiological evidence for the transmission of other enteric viruses by the water route (Mosley, 1967; IAWPRC Study Group on Water Virology, 1983). Lack of data may be due to the fact that only hepatitis must be reported to the Centers for Disease Control (CDC). Moreover, even with the tens of thousands of cases of waterborne infectious hepatitis that occurred in Delhi in 1955 and 1956 (Viswanathan, 1957) [perhaps non-A hepatitis virus (Wong et al., 1980)], the total number of cases of infectious hepatitis proven to be waterborne is a small fraction of the total number of reported cases. Nonetheless, there is potential for disaster in the water route of viral transmission (Mosley, 1967). The massive Delhi outbreak is grim evidence of the hazard. Moreover, the lack of reported outbreaks of water-related enteric virus infections may be attributable to the inability of conventional surveillance and other epidemiologic techniques to detect water transmission (Goldfield, 1976). Goldfield, for example, made the following notation:

> "Outbreaks produced by an agent that is regularly or frequently associated with an easily recognized uniform clinical syndrome such as measles or infectious hepatitis will be detected much more easily than one produced by a virus associated with variable, protean nonspecific clinical signs of disease as is often the case with adenoviruses and picornaviruses. It is no accident that typhoid and cholera are the classic examples of waterborne disease and that infectious hepatitis is the sole virus disease thus far incriminated with any regularity in water transmission. The adenoviruses and picornaviruses, for example, may be transmitted by potable water, but the high level of immunity in many age groups, the probably high inapparent infection-to-disease ratios and the variable nonspecific symptoms associated with human infection by most of these agents would make waterborne outbreaks more difficult to detect. Unless massive contamination of drinking water occurred with one of these viruses, I doubt if such contamination would exceed the threshold of recognition by the usual disease surveillance technics. Therefore, it seems unwise at this time to ascribe too much significance to the absence of epidemiologic evidence relating viruses other than that of infectious hepatitis to waterborne outbreaks of human disease."

Melnick (1976, 1978), reflecting on Goldfield (1976), subsequently commented as follows:

> "The general public, and some large part of the medical community, tend to have a distorted impression of disease surveillance. Over the years, many have been led to believe that a foolproof system exists for the recognition of disease outbreaks, which are then studied and controlled by public health authorities. To the public, the absence of reported outbreaks provides reassuring evidence of a safe environment. Unfortunately, this reassurance is often without substance."

Seasonal Distribution of Viruses in Water. Viruses in sewage have been detected in numbers ranging from <100 to >100,000 cell culture infective units per liter of sewage. Generally, in temperate climates the numbers are greater in the warmer months and lesser in the colder months. These numbers reflect the infection and excretion patterns for most viruses in the community. Hepatitis A infections and rotavirus infections are more prevalent in the colder months, but hepatitis A virus and rotaviruses are not as easily recovered from the water environment as most other enteric viruses. Many of the adenoviruses are also transmitted more frequently in colder weather, but the cell culture systems used for recovering viruses from environmental waters are not as sensitive to adenoviruses as they are to most of the enteroviruses. Other viruses, such as the Norwalk and Norwalk-like viruses, have not been detected in the water environment because they have not yet been sought, or because methods for their detection in water are not available.

Waterborne Outbreaks of Viral Diseases in the United States.

Sizes of Outbreaks -- Lippy and Waltrip (1984) reported that from 1946-1980 viruses were responsible for 11.8% of the waterborne outbreaks of disease in the United States. During this period, 21.7% of the outbreaks were attributed to bacteria, 7.3% to a chemical etiology, and 7.1% to protozoans. The etiology of 52.1% of the outbreaks was unknown. Of the clearly viral outbreaks that occurred from 1946-1982 (Table II-2), 73 were infectious hepatitis [most were probably hepatitis A (enterovirus 72)], 17 were caused by Norwalk virus, at least one was caused by a rotavirus, and one was caused by a poliovirus (Kaplan et al., 1982; CDC, 1983; Lippy and Waltrip, 1984; Gerba et al., 1984; Sobsey et al., 1984). Of the reported outbreaks of acute nonbacterial gastroenteritis that occurred between 1976 and 1980, 42% were caused by Norwalk virus (Kaplan et al., 1982). It is likely that many of the outbreaks of unknown etiology were viral (Haley et al., 1980). Among the 38 Norwalk virus outbreaks reported through 1980, 31 were common source. Thirteen of the 17 outbreaks in which a vehicle was implicated were waterborne. The remaining four were foodborne (Kaplan et al., 1982). Drinking water and recreational water outbreaks of Norwalk virus-induced disease in the United States are shown in Table II-3. Drinking water outbreaks of rotavirus-induced disease worldwide are shown in Table II-4.

Numbers of Illnesses -- Although most of the waterborne disease outbreaks in the United States reported from 1946-1982 were produced by hepatitis viruses, the Norwalk and Norwalk-like viruses produced the greatest number of illnesses. There were 2415 cases of hepatitis during 73 outbreaks and 3692 cases during 17 outbreaks of Norwalk virus-induced gastroenteritis (Lippy and Waltrip, 1984).

TABLE II-2

Waterborne Outbreaks of Viral Etiology in the United States[a]

Virus	Years	No. of Outbreaks	Cases of Illness
Infectious hepatitis	1946-1982	73	2415
Norwalk	1975-1982	17	3692
Rotavirus	1976-1982	2[b]	2169
Poliovirus	1946-1982	1	16

[a] Source: Abstracted, revised and modified from Kaplan et al. (1982), CDC (1983), Lippy and Waltrip (1984), Gerba et al. (1984) and Sobsey et al. (1984)

[b] One outbreak, Colorado, 1976, cited in Gerba et al., 1984, is based on limited data and has not been recognized as a rotavirus outbreak by the CDC (Harris et al., 1983).

TABLE II-3

Drinking Water and Recreational Water Outbreaks of Norwalk Virus-Induced Disease in the United States*

Date	Location	Number Ill	Water Supply	Remarks
		DRINKING WATER		
1975	Cruise Ship	6	Tank	NR
1978	Pennsylvania	350	Drinking water with insufficient chlorination Well water	Attack rate 17-73%, summer camps - two outbreaks
1978	Washington	600	Well, 51.4 m deep	Attack rate 72%
1979	California	30	Sprinkler irrigation system not for human consumption underground creek	Creek water with viral titer of 14/100 mℓ
1979	North Carolina	145	Well	Attack rate 78%
1979	Cruise Ship	5	Tank	NR
1980	California	10	NR	Recreational area
1980	Pennsylvania	5	NR	Restaurant
1980	Georgia	14	NR	Community
1980	New York	5	Well	Camp
1980	Maryland	126	Well 95 ft. deep	Attack rate 64%, disinfected water, no coliforms

TABLE II-3 (cont.)

Date	Location	Number Ill	Water Supply	Remarks
		DRINKING WATER (cont.)		
1981	North Carolina	146	Well water	Coliform and fecal coliform found in 1 of 2 wells
1982	Georgia	1500	Spring	Attack rate 72%
1982	Georgia	500	Springs and well	Spring possibly contaminated from rainfall events from the surface
1982	Alabama	55	Well	Distribution system deficiency
1982	California	25	Storage tank	Treatment deficiencies
1982	California	170	Broken main	Distribution system deficiency
		RECREATIONAL WATER		
1977	Ohio	7	Swimming pool	NR
1978	Pennsylvania	350	Recreational area	NR
1979	Michigan	121	Park lake	Geometric coliform density within acceptable U.S. EPA limits-clearly documented secondary attack rate

*Source: Modified and revised from CDC (1983) and Gerba et al. (1984)

NR = Not reported

TABLE II-4

Drinking Water Outbreaks of Rotavirus-Induced Disease[a]

Date	Location	Number Ill	Remarks
1976[b]	Colorado	418	Rotavirus isolated Snow Mt. outbreak
1977	Sweden	3172	NR
1980[c]	Norfolk Island	NR	Contamination of groundwater supply
1980	Brazil	~900	NR
1981	Russia	173	NR
1981	Colorado	1761	Contaminated community supply
1981	East Germany	4% of population	Flood water contamination of wells
1982	Israel	~2000	Reservoir contaminated by children

[a] Source: Modified and revised from CDC (1982b) and Gerba et al. (1984)

[b] Outbreak not reported as rotavirus-induced by CDC (Harris et al., 1983)

[c] Supporting data limited (Gerba et al., 1984)

NR = Not reported

Sources of Contamination -- Eleven of 22 outbreaks of infectious hepatitis that occurred from 1946-1982 in community systems* resulted from contamination of the distribution systems (Table II-5) (Craun, 1981; CDC, 1982a; Sobsey et al., 1984). Cross connections and backsiphonage were the major factors that brought about the contamination. Untreated groundwater was the major culprit in outbreaks of infectious hepatitis attributed to water supplies other than those classified as community supplies. The water sources incriminated in outbreaks of Norwalk virus-induced gastroenteritis are shown in Table II-6.

Outbreaks of Infectious Hepatitis -- Mosley (1967) listed 50 published reports in which outbreaks of infectious hepatitis had been attributed to contaminated drinking water. These reports were of worldwide occurrences and dated from 1895-1964. Mosley judged that data in at least 30 of these outbreaks was sufficient to implicate water as the vehicle of transmission and that the data on many of the other outbreaks were sufficient to accept a water vehicle at least as a plausible hypothesis. The total number of cases in all of the 50 reported outbreaks was about 33,500 -- a small fraction of the total number of cases of infectious hepatitis that occurred during that period. There is no way to estimate undetected and unreported cases accurately.

---

*Community (municipal) water supply systems are defined in the Safe Drinking Water Act (PL93-523) as publicly or investor-owned supplies that serve large or small communities, subdivisions, or trailer parks and that have at least 15 service connections or regularly serve at least 25 year-round residents. Noncommunity (semipublic) water systems are those that serve transitory populations. These systems supply institutions, campers, parks, hotels, industries and service stations with their own water supplies. Individual water systems are those that are used by single residences in areas where there are no municipal systems or by people traveling in poorly populated areas.

TABLE II-5

Waterborne Outbreaks of Infectious Hepatitis (probably hepatitis A) in the United States, 1946-1982*

Outbreak Cause	No. of Outbreaks in Community Systems	No. of Outbreaks in Noncommunity and Industrial Systems
Contaminated, untreated surface water	1	11
Contaminated, untreated groundwater	6	28
Inadequate or interrupted disinfection	3	5
Contamination in distribution systems	11	2
Data insufficient to classify	3	2
Total	24	48

*Source: Modified from Craun (1981), CDC (1982a) and Sobsey et al. (1984)

TABLE II-6

Waterborne Outbreaks of Norwalk Virus-Induced Disease*

No. of Outbreaks	Water Source
2	Community public water systems
7	Noncommunity public water systems
2	Stored water (cruise ships)
2	Recreational swimming water

*Source: Abstracted and revised from Kaplan et al. (1982)

Included in the Mosley (1967) report was an outbreak of waterborne hepatitis in Delhi, India in 1955 and 1956 that produced thousands of cases of illness. The outbreak, as with all waterborne outbreaks of hepatitis, had long been assumed to be of hepatitis A etiology. Recently, however, a report has been published suggesting that this massive waterborne outbreak of hepatitis was neither hepatitis A nor hepatitis B and thus was of non-A, non-B etiology (Wong et al., 1980). Subsequently, a number of other reports have been published suggesting that other waterborne outbreaks of hepatitis in India were also of non-A, non-B etiology (Khuroo, 1980; Wong et al., 1980; Pavri et al., 1982). Certain characteristics of these outbreaks including epidemicity, apparent fecal-oral transmission, high mortality among pregnant woman, and a high rate of miscarriage have suggested that the etiological agent(s) involved is not the same as the non-A, non-B agent involved in blood and blood product-related transmission. Some evidence exists that suggests that the etiological agent(s) of these outbreaks is the same as the etiological agent(s) of outbreaks that have occurred elsewhere (Purcell, 1982).

Many outbreaks of infectious hepatitis have occurred since the Mosley (1967) report. A recently reported waterborne outbreak of infectious hepatitis in the United States occurred in Georgetown, Texas in 1980 (Hejkal et al., 1982). The hepatitis outbreak was heralded by an outbreak of gastroenteritis. The outbreaks were thought to have been caused by large amounts of human fecal matter in the vicinity of the city's well water supply, fecal matter that may have been washed by heavy rains through a fault and through limestone formations into the wells. Illnesses were associated with drinking water from the wells that had been treated only by chlorination. Cases of hepatitis began to appear about a month after the first cases of gastroenteritis appeared. A total of 36 cases of hepatitis occurred over a period of about a month. About 7900 cases of gastroenteritis occurred in the 10,000 people living in the affected area. Samples of well water taken 2-3 weeks after onset of the outbreak of gastroenteritis yielded hepatitis A antigen and coxsackieviruses B2 and B3. Samples of tap water taken at the same time yielded coxsackievirus B3. The hepatitis A antigen and the two coxsackieviruses were also recovered from sewage. The tap water contained 0.8 mg/ℓ of total chorine. Rotaviruses were recovered from sewage samples but not from the stools of six patients tested nor from well and tap water. Coxsackievirus B3 has been associated in the past with diarrhea in children. Routine bacteriological tests for coliforms on the tap water throughout the period of the outbreaks were negative.

Outbreaks of Poliomyelitis -- Only eight reported outbreaks of poliomyelitis have been attributed to water transmission through the years. Convincing evidence in support of a water route exists, moreover, for only one of these outbreaks (Mosley, 1967). That outbreak occurred in Huskerville (Lincoln), Nebraska in 1952 in temporary housing units. Wide fluctuations had occurred in water supply pressure in these units, and vaccuum breakers were missing in some toilets (Bancroft et al., 1957). Seventeen cases of paralytic poliomyelitis and 28 cases of nonparalytic illness occurred in a 5-week period during the months of June and July.

Outbreaks of Gastroenteritis -- Technology developed in recent years has permitted the identification of viral agents responsible for numerous outbreaks of waterborne gastroenteritis. Rotaviruses are the major etiological agent of infantile diarrhea and have been implicated in several waterborne epidemics of gastroenteritis worldwide (Gerba et al., 1984). Norwalk virus has been implicated in 17 outbreaks of waterborne gastroenteritis (Craun, 1981; Kaplan et al., 1982; Gerba et al., 1984). Norwalk-like viruses have been implicated in several other waterborne outbreaks of gastroenteritis. The Norwalk virus was first recovered during a suspected waterborne gastroenteritis outbreak in Norwalk, Ohio in 1968.

Gastroenteritis at recreational beaches in New York City, Lake Pontchartrain, Louisiana, and in Boston may also have been caused by rotaviruses or by Norwalk or Norwalk-like viruses (Cabelli et al., 1982). The occurrence of gastroenteritis at these beaches correlated with the numbers of enterococci in the bathing waters but not with the numbers of coliforms.

Summary

Enteric viruses that multiply in or near the GI tract of man are excreted into domestic sewage by infected individuals. Subsequently, this sewage is discharged (with or without treatment) into rivers and streams. Enteric viruses comprise more than 120 different viruses. Sewage, somewhat diluted in the river, flows downstream and impacts recreational waters, water intakes, and perhaps estuaries that support shellfish beds. Sewage discharged to the land may impact groundwaters. Viruses have been recovered from river waters collected many miles downstream of outfalls, at water intakes, and from groundwaters impacted by discharges of domestic sewage to the land.

The enteric viruses comprise the enteroviruses, reoviruses, rotaviruses, adenoviruses, and a number of small round viruses that include Norwalk virus, Norwalk-like viruses, caliciviruses, astroviruses and coronavirus-like agents. These viruses are responsible for many human diseases including poliomyelitis, aseptic meningitis, infectious hepatitis, herpangina, pleurodynia, gastroenteritis and rashes.

With the exception of one outbreak of poliomyelitis, only infectious hepatitis was proven to be waterborne until a decade or so ago. In the past 10 or 15 years, however, at least six waterborne outbreaks of rotavirus-induced disease have been documented worldwide (one in the United States), 17 outbreaks of waterborne Norwalk virus-induced gastroenteritis have been reported in the United States alone, and a number of outbreaks of gastroenteritis induced by Norwalk-like viruses have also been reported. Evidence suggests that most of the nonbacterial waterborne gastroenteritis reported in earlier years was produced by Norwalk and Norwalk-like viruses. Moreover, it is probable that reported outbreaks constitute only the tip of the iceberg, for without the existence of surveillance systems designed to detect disease outbreaks, most epidemics, especially waterborne epidemics, probably escape detection.

Although many outbreaks of waterborne disease are small, they can be large and devastating. For example, the New Delhi outbreak of infectious hepatitis in 1955 and 1956 resulted in tens of thousands of illnesses, and a recent waterborne outbreak of gastroenteritis in Georgetown, Texas resulted in almost 8000 illnesses in a population of ~10,000.

Most waterborne outbreaks of infectious hepatitis reported in the United States for the years spanning 1946-1982 were associated with noncommunity water supply systems and resulted from consumption of untreated groundwater. Outbreaks of Norwalk virus-induced gastroenteritis that have been reported in the United States were also associated with noncommunity water supply systems.

III. OCCURRENCE IN WATER

Viruses in Environmental Waters

The viruses of virtually all species undoubtedly occur in environmental waters. These include the viruses of man, animals (domestic and wild), plants, fish, insects and other invertebrates, and bacteria. Other than viruses originating in man, not many are important to man directly and few are known to infect him. However, some viruses may affect man indirectly. For example, man might be affected economically if a disease outbreak among farm animals was transmitted through water. This chapter will describe viruses that occur in environmental waters and that may be infectious to man.

Viruses of Man. The viruses of man that may be present in sewage probably include almost all viruses that infect man. All of the viruses that are excreted with feces and urine become a part of sewage. Additionally, viruses of the respiratory tract and saliva, which are swallowed (or spit out), and viruses that produce viremia (and which thereby occur in menstrual blood) or that occur in the vagina, may also occur in sewage. Even the agents of genital herpes and of AIDS probably occur in sewage. Other than the viruses that enter sewage with feces, few viruses have been sought in the water environment and few have been detected. Many viruses present in sewage can conceivably occur in sludges, recreational waters and drinking waters.

Viruses of Animals. Since domestic animals in municipalities generally defecate and urinate on the streets, storm water runoff probably teem with viruses of these species. Since most municipal sewers are combined sewers, domestic sewage probably also teem with these viruses. The same situation must prevail for rats, mice, squirrels and other wildlife that dwell in cities. Rivers and other streams, as they take runoff from the land, undoubtedly receive large numbers of viruses from farm and wild animals. Through the years, there has been only occasional concern about possible water transmission of viruses from feedlot to feedlot or from farm to farm. Although there are viruses that infect both man and animals, municipalities found little need for concern for the possible transmission of viruses from animals to man through the water route.

Viruses of Fish. Viruses of fish are transmitted to the water environment, but they are not known to infect man. However, a calicivirus recently recovered from an opaleye fish can infect swine experimentally (Smith et al., 1980). With fish viruses, the possible economic impact from diseases of commercially grown fish would seem to be the major concern. In recent years, for example, the IPN virus has caused extensive losses of fish fry on trout farms and in other hatcheries in England leading to concern that the disease might spread to the environment through sewage (Slade and Ford, 1983).

Viruses of Plants. Since many plants are aquatic, it is likely that many plant viruses exist in environmental waters and that water plays some part in their transmission. The viruses of terrestrial plant life undoubtedly are washed into waterways, and these too probably abound in water. Many plant viruses require aphid or nematode vectors, and aquatic nematodes could conceivably carry plant viruses into sludges or effluents, which if used for agricultural purposes might pose a problem (Slade and Ford, 1983). No evidence for significant transmission of this sort is known.

Bacterial Viruses. Since bacteria abound in environmental waters, bacterial viruses abound there also. Bacterial viruses are not directly harmful to man, but it is possible for these viruses to transmit genetic material from one bacterium to another, a process known as transduction. Thus, it is possible for a bacterial virus to transmit genetic material producing resistance to certain antibiotics from a nonpathogenic species of bacteria that are resistant to those antibiotics to a nonresistant pathogenic species, thereby rendering the initially nonresistant pathogen resistant. Other such transformations with hazardous implications for man may also occur, but actual hazards have yet to be clearly documented.

Bacterial viruses may also serve a useful purpose. Viruses that infect indicator bacteria may themselves be indicators of pollution, although an acceptable test system to utilize bacterial viruses as indicators has not yet been developed. Moreover, bacterial viruses are usually more resistant to disinfectants than indicator bacteria, and efforts to utilize such viruses as indicators of the effectiveness of disinfection in treatment processes may have some merit.

Viruses in Sewage

Although only 3 decades ago viruses were only infrequently recovered from raw domestic sewage, the technology for achieving such recovery has vastly improved. Today, viruses can always be recovered from such sewage.

Sewage is the source of most of the viruses of human origin found in the water environment. Viruses occur in sewage throughout the year (Berg, 1973a,b). Most of these viruses occur in greatest concentrations during the warmer months of the year. Hepatitis A virus (enterovirus 72) and rotaviruses, neither of which is easily detected in sewage, and some adenoviruses, which are not often tested for in sewage, are more frequently recovered in the cold seasons than in the warm seasons, because these viruses produce more infections in the community during cold weather than during warm weather. However, the live-poliovirus vaccination programs, supported by conventional pediatric practice, maintain a high infection rate in the younger members of the community and equalize the numbers of viruses in sewage in all seasons.

Many viruses are removed or inactivated by treatment processes before sewage is discharged to the water environment. But even terminal disinfection, largely with the chloramines that form quickly when chlorine or hypochlorite ion is added to sewage effluent, still permits the survival and discharge of large numbers of viruses to the rivers and streams serving as the raw water sources for many of this Nation's major communities.

Sewage treatment plants are the major collection centers for the domestic liquid wastes of the largest communities of this country. These plants represent the greatest opportunity available to deal with problems posed by discharged effluents to downstream river communities. No community that practices good sanitation constructs its water intakes downstream of its own sewage discharges, and thus, the discharging communities create no hazard for themselves. Communities downstream have no choice other than to construct their water intakes downstream of the sewage discharges of polluting communities situated upstream.

Factors that Affect the Numbers of Viruses in Sewage. The numbers of viruses in the domestic sewage of any given community depend on several factors as listed below:

Age Skew in the Community -- Most viral infections occur primarily in the younger members of the community, who are most susceptible and have the highest rate of transmission. Thus, it is in communities where age distribution is skewed towards grade school and preschool children that the greatest numbers of viruses in sewage are most likely to occur. Moreover, in these communities live poliovirus vaccine programs have the greatest application, and it is likely that the numbers of excreted viruses entering domestic sewage may be significantly increased.

Immunologic Status of the Community -- The immunologic status of the community is dependent directly on the epidemic and endemic infection history of the community and on the age distribution of the community's inhabitants. Most viruses, once they have circulated throughout a community, leave behind resistance to reinfection in all individuals who have been infected. This refractory state may not be permanent, but reinfection is not readily achieved for at least a period of time. The Norwalk virus, however, seems to be an exception to these normal patterns of immunity acquisition (Koopman et al., 1982).

Introduction of the Virus into the Community -- For a virus to circulate within a community and cause GI infections, which inturn contribute to viruses in the community's sewage, the virus must first gain entrance into the community. Thus, the virus must be transported by an individual, most likely by a school age or preschool age child, or through a vehicle such as water or food from a community in which the virus is epidemic or endemic into another community in which it is not.

Sanitary Level of the Community -- Transmission rates for enteric viruses within a community are probably related to the availability of water in the community. Water availability and water quality are direct reflections of the sanitary status of the community (CDC, 1980).

In metropolitan areas, the larger the size of the lower socioeconomic segment of a community, the larger will be the number of viruses in the community's domestic sewage.

Dilution of the Sewage -- The availability of water probably has a direct effect on the virus excretion rates in the community (CDC, 1980), and it also affects the concentration of viruses in sewage. In areas where large quantities of industrial sewage are mixed with a community's domestic sewage, the dilution considerably reduces the concentration of viruses in the combined sewage. Similarly, in areas where water is in short supply, water usage conventions may also bring about increased concentrations of viruses in sewage.

Chemical Composition of Sewage -- Toxics, especially from industrial wastes, may reduce the numbers of viruses in sewage, because viruses are subject to chemical inactivation. Viruses, however, can be protected by a favorable chemical environment. For example, chemicals that maintain a favorable pH level in sewage are protective.

Biological Flora of Sewage -- Protozoans and bacteria may engulf viruses. They may also destroy viruses with antiviral agents they produce. Viruses, however, may also adsorb to these biota and be protected to some degree from noxious agents in the environment. Viruses that are ingested by nematodes may be protected from chlorination (Chang et al., 1960).

Solids in Water -- Viruses adsorb readily onto solids in water. Adsorption to solids is a major mechanism for virus removal by sewage treatment processes. Adsorption of viruses onto solids is also a mechanism for protection, because viruses are protected from noxious agents by nonliving solid materials just as they are by solid biota to which they adsorb. Moreover, viruses that become entrapped within cellular solids, fecal solids, or other solids are protected from the noxious environment. Such viruses may be difficult to detect.

Climate -- The circulation of many enteric viruses in the community increases during the warmer months. Thus, in areas of the country where temperatures are higher, the numbers of enteric viruses in sewage should be greater than the numbers in the sewage of communities in colder sections of the country. This is probably so even though viruses are more rapidly inactivated in warmer water than in colder water. The differences in numbers reflects the difference in the extent of circulation of these viruses in the communities in these areas. One might expect the reverse to be true with viruses that increase in circulation in colder weather. However, little quantitative data are available that demonstrate these differences.

Recovery of Viruses from Sewage.

Raw Sewage -- The numbers of viruses recovered from raw sewage in the United States range from ~50-250 PFU/ℓ. The numbers of viruses in raw sewage may be much greater in arid areas. In Israel, for example, in a study that ranged over an 11-month period, Buras (1976) recovered more than one million PFU of viruses/ℓ in a sewage sample collected during a warm month (Table III-1). The smallest recoveries she experienced (during January), averaged >21,000 PFU/ℓ. The large numbers of viruses in these sewage samples permitted Buras to bypass the concentration procedures normally required when viruses are recovered from sewage and from other environmental waters. Concentration procedures are inefficient, and viruses are partially lost when they are concentrated. Concentration of viruses in Buras' sewage samples would probably have reduced the numbers recovered by a factor of at least two.

Primary Effluents -- Primary settling probably removes more viruses from raw sewage than most data suggest. Studies over the years have suggested removals from 0 to 60 or 70%, but these studies did not consider the numbers of viruses entrapped within fecal solids and cellular debris. These viruses are not counted because only the liquid part of the sewage is

TABLE III-1

Viruses in Sewage in an Arid Land*

	Viruses Recovered (PFU/ℓ)	
Month	Average	Maximum
March	133,000	225,000
April	211,500	425,000
May	492,000	500,000
June	291,300	660,000
July	280,000	1,060,000
August	175,000	600,000
September	175,000	580,000
October	32,500	100,000
November	84,000	300,000
December	17,000	40,000
January	21,300	105,000

*Source: Abstracted and revised from Buras (1976) assayed. Thus, there are undoubtedly many more viruses in raw sewage than studies have shown, and many more viruses are probably removed with settled solids from primary effluents as well (Berg, 1973b).

In any event, the number of viruses that remain in effluents after settling is still high. Table III-2 shows that in eight primary effluent samples taken from three different treatment plants, the number of viruses remaining in each effluent after settling still ranged in the hundreds/ℓ.

Activated Sludge Effluents -- Activated sludge is the best of the biological sewage treatment processes. In laboratory studies, activated sludge removed >90% of the viruses remaining in primary effluents, although such removals are not achieved in the field unless plants are well operated (Berg, 1973b).

In a study of activated sludge treatment plants in four cities, Moore et al. (1978) found that >90% of the viruses in the mixed liquor suspended solids were solids-associated, thus accounting for the mode of removal of viruses from activated sludge effluents. Table III-3 shows the high removal rate of viruses by activated sludge in treatment plants of the four cities (Austin, Chicago, Butte and Portland). Table III-4 shows that an oxidation ditch also displaced 90% of the viruses to the sludge. These tables also show that many viruses still remained in the effluent (Moore et al., 1978; Sorber, 1983).

TABLE III-2

Viruses in Primary Sewage Effluents*

Treatment Plant	Test No.	Primary Effluent (PFU/ℓ)
A	1	192
	2	427
	3	178
	4	422
	5	109
	6	222
B	1	210
C	1	185

*Source: Abstracted and revised from Berg et al. (1978)

TABLE III-3

Distribution of Enteroviruses in Activated Sludge Aeration Basins[a]

Treatment Plant	Location of Treatment Plant	Mixed Liquor Suspended Solids (mg/ℓ)	Viruses Recovered (PFU/ℓ)		% of Viruses Associated With Solids
			Solids[b]	Liquid	
1	Austin	1800	950	90	93
		2100	600	40	95
		1700	450	70	91
2	Chicago		202	40	83
			190	2	99
			279	5	98
3	Butte	1560	720	57	93
4	Portland	2780	500	34	94

[a] Source: Abstracted and revised from Moore et al. (1978)

[b] PFU of viruses obtained from collected solids from 1 ℓ of mixed liquor

TABLE III-4

Removal of Viruses from Raw Sewage by an Oxidation Ditch and by a Trickling Filter[a]

Material Sampled	Sample No.[b]									
	1	2	3	4	5	6	7	8	9	10
Raw sewage (PFU/ℓ)	9.1	6.8	30	8.0	7.0	5.6	70	780	910	34
Oxidation ditch effluent (PFU/ℓ)	0.7	0.3	2.0	1.3	0.5	2.2	1.8	1.0	5.7	1.2
% Reduction	92	96	94	84	93	61	97	99.9	99.4	96
Trickling filter effluent (PFU/ℓ)	25	0.7	1.6	1.1	2.1	1.9	21	320	140	7.0
% Reduction	73	90	95	86	71	66	70	59	85	79

[a] Source: Abstracted and revised from Moore et al. (1981)

[b] Five-day composites

Trickling Filter Effluents -- The effectiveness of trickling filters for removing viruses from sewage effluents varies widely (Berg, 1973b). Well-designed, well-operated trickling filters are effective in removing viruses from effluents, but not as effective as the activated sludge process. Table III-4 shows that although trickling filters removed ~77% of the viruses from raw sewage, the extended aeration activated sludge process removed ~91% of the viruses from the same effluent (averages not shown) (Moore et al., 1981; Sorber, 1983). The apparent poor removal of viruses sometimes experienced with trickling filters has been attributed to the disintegration of fecal material in the filters and the consequent release of virus particles that had not been counted in the influent (Sorber, 1983).

Stabilization Ponds -- Stabilization ponds, like trickling filters, are only sometimes effective in removing viruses from raw sewage. Short-circuiting may be responsible for poor virus removal. When viruses are effectively removed, elevated pH levels of the pond water (brought about by the metabolism of algae), detention time, sunlight, biological adsorption and engulfment, and adsorption to solids play a role (Berg, 1973b, Sorber, 1983).

Advanced Waste Treatment Systems (Water Renovation) --

Coagulation and Flocculation. The most important of the advanced waste treatment systems and the most effective for removing viruses from effluents is chemical coagulation and flocculation. For this purpose, $AlCl_3$ (alum), $FeCl_3$ (ferric chloride), and $Ca(OH)_2$ (lime) are about equally effective. Moreover, long contact with lime, because of the high pH that lime induces, is virucidal (Berg, 1973b; Sproul, 1976). For example, in secondary effluents, flocced with 500 mg/ℓ of $Ca(OH)_2$, adjusted to pH levels of 10.1, 10.8 and 11.1 with HCl (hydrogen chloride), and seeded with poliovirus 1, the virus was unaffected after 2 hours at the lowest pH level, 50% of the virus was inactivated after 2 hours at the middle pH level, and 99.4% of the virus was inactivated after 2 hours at the highest pH level (Berg et al., 1968).

Sand Filtration. Sand filtration is of varying effectiveness in removing viruses from coagulated, flocced effluents. The extent of removal increases as the rate of filtration decreases. The extent of removal is even greater when a partially settled, flocced effluent is passed through the sand filter. Apparently, the carryover floc itself serves as a virus adsorbent and retains viruses not trapped in the sand.

Carbon Filtration. Effluents that have been coagulated and filtered through sand may be filtered through carbon to remove organics. Removal of viruses from effluents by carbon filtration vary between ~20 and 50%. Many viruses adsorbed to carbon columns eventually elute as adsorptive sites on the carbon become saturated, and as moieties come through that have greater affinity for receptor sites on the carbon than the viruses have. In this situation, viral breakthroughs occur and the number of viruses in the carbon column effluent may be greater than the number of viruses in the influent to the column (Berg, 1973b). Moreover, some viruses may adsorb more efficiently than others. For example, rotaviruses may not adsorb as efficiently as polioviruses (Gerba et al., 1984).

__Disinfection__ -- Disinfection may follow secondary treatment or advanced waste treatment. The effectiveness of disinfection in destroying viruses depends on the extent to which the treatment applied to the effluent removes materials that interfere with disinfection. Generally, with more treatment before disinfection, the disinfection process is likely to be more effective. Thus, an effluent with little turbidity, organic material, and ammonia (if chlorine is the disinfectant) is relatively easy to disinfect. An effluent that contains solids that protect viruses, organics that react with the disinfectant, or ammonia (if chlorine is the disinfectant) that forms chloramines (much slower virucides than the free chlorines) is relatively difficult to disinfect (Gerba, 1980).

Some viruses are inactivated more rapidly by disinfectants than others. The simian rotavirus, SA 11, for example, is inactivated more rapidly by free chlorine than hepatitis A virus or polioviruses (Grabow et al., 1983).

__The Final Effluent__ -- Few domestic sewages in this country today receive more than one form of secondary biological treatment before discharge to rivers or streams, and many sewages do not yet receive that much treatment even though the law requires it. Advanced waste treatment processes are usually reserved for sewages destined for immediate reuse (rare in this country today). A renovated sewage can be disinfected effectively if it contains little turbidity, organic material, and ammonia (if chlorine is the disinfectant). Disinfection, as commonly practiced in this country for primary and secondary effluents, permits many viruses to escape into rivers and streams, which impacts downstream recreational areas, water intakes, and shellfish beds as the following sections attest.

Viruses in Surface Waters

Sewage treatment plants along rivers and streams continuously discharge viruses to those watercourses. Septic tanks and sewages discharged by farms, small towns, and isolated houses add to the total viral loading in surface waters.

Survival of Viruses in Surface Waters.

The Tanana River Study -- Viruses can survive for long periods in surface waters. Dahling and Safferman (1979) traced the flow of viruses originating at a single sewage treatment plant in Alaska along the Tanana River, and recovered 300 km downstream ~30% of the numbers of viruses they had detected at the sewage treatment plant itself. The temperature of the river was 0°C. The flow rate of the river was such that 7 days were required for the viruses to cover that distance. The Tanana River courses through relatively desolate arctic wasteland and during the frozen winter, when this study was done, there was no pollution of the watercourse from sources other than the treatment plant. Although the situation along this watercourse is not typical of those throughout most of the United States, the study demonstrated the capability of the virus to traverse long distances and survive at least at cold temperatures.

During the winter months, the temperatures of the waters in many parts of the United States do not differ greatly from the temperatures of the Tanana River. Although industrial pollution undoubtedly inactivates some viruses in the more developed areas of this country, the constant recharge from many municipalities, towns, farms, and other point sources serves to continuously restock the waterways with viruses.

Role of Solids in Virus Survival -- For more than a decade, it has been apparent that viruses adsorb to solids in the water environment, and that the number of viruses adsorbed to solids in water usually exceeds the number that remain free in the ambient waters (Berg, 1973a). Viruses are colloidal matter and adsorb readily to many kinds of solids in water. Such adsorption begins in or near the GI tract of man where most of the viruses in sewage originate and may occur anywhere else where viruses are found. The great affinity that viruses have for solids is shown in Table III-5 (Block et al., 1979). This table shows that poliovirus 1, mixed with activated sludge, adsorbed to sludge particles rapidly and extensively. Ninety percent of the virus particles were adsorbed within 1 minute of contact. In 5 minutes, 99.9% of the poliovirus had adsorbed, and in 60 minutes, 99.997% of the virus had adsorbed. If such rapid and extensive adsorption is extrapolated to raw sewage or to fecal material, and one judges by the numbers of viruses remaining free in the liquid part of the effluent, it would seem that the numbers of viruses present in those materials would be orders of magnitude greater than the numbers actually reported. Rotaviruses, however, do not adsorb to activated sludge solids or to other solids as extensively as enteroviruses (Farrah et al., 1978; Steinmann and Havemeister, 1982; Gerba et al., 1984). Rotaviruses do not adsorb as well to soil either (Goyal and Gerba, 1979).

Table III-6 shows the numbers of viruses recovered from the waters of Galveston Bay and from the sediments obtained from those waters. In 3 of 4 samples, the numbers of viruses eluted from the sediments were 10 or more times greater than the numbers present in the waters (LaBelle et al., 1980). Since the techniques used to elute viruses from sediments are of low

TABLE III-5

Adsorption of Poliovirus 1 to Activated Sludge Solids*

Time (minutes)	Viruses Adsorbed (%)
1	90
5	99.9
30	99.99
60	99.997

*Source: Abstracted and revised from Block et al. (1979)

TABLE III-6

Recovery of Viruses from Bay Waters and Sediments[a]

Material Tested	Sample No. 1	2	3	4
Seawater[b]	6	528	5	30
Sediment[c]	200	140	50	480

[a] Source: Abstracted and revised from LaBelle et al. (1980)

[b] 20 ℓ

[c] Sediment from 20 ℓ

efficiency, it would seem that the percentages of viruses in the sediment portions of bay water samples are even greater than the table suggests.

Viruses adsorbed to solids in a sewage treatment plant are likely to be removed with the sludge. Viruses that remain free and reach a watercourse with discharged sewage may adsorb to suspended matter and eventually become a part of sediment or be carried downstream as part of the suspended solids. In either event, viruses adsorbed to solids are protected from the environment. Viruses encased in cellular debris or in detritus receive a considerable amount of protection. Studies have shown that most of the viruses in surface waters are solids-associated (Berg, 1973a), that the solids often become a part of the sediment (DeFlora et al., 1975; Goyal et al., 1978; LaBelle et al., 1980), and that viruses adsorbed to and within solids are protected from inactivation by biological, chemical and physical factors in the environment (DeFlora et al., 1975; Gerba and Schaiberger, 1975; Smith et al., 1978; LaBelle and Gerba, 1980).

Viruses adsorbed to solids that have become part of the sediment of a waterway may be swept up from the bottom by heavy rains or by spring runoff and carried to downstream recreational areas and water intakes. Solids-adsorbed viruses may also be swept into shellfish beds and engulfed by shellfish. Bioaccumulation of viruses by shellfish is favored by solids-association (Hoff and Becker, 1969; Metcalf et al., 1979).

Role of Physical, Chemical and Biological Factors in Virus Survival -- Block (1983) listed a number of physical, chemical and biological factors that affect the survival of viruses in environmental waters. The factors playing a role in viral survival in surface waters play the same role in sewage. Most of these factors are affected by whether the virus is solids-adsorbed or free in the water.

-. Physical Factors. Among the physical factors affecting the survival of viruses are light, temperature, hydrostatic pressure, adsorption and aggregation (Block, 1983).

Light has long been known to be virucidal, especially light that is in the ultraviolet range. It is absorbed by viral proteins and nucleic acids, resulting in a change in the configuration of the proteins and some disintegration in the nucleic acids (Bitton, 1978). Other mechanisms of viral inactivation are also possible. Viruses aggregated or adsorbed on solids, are partially protected from exposure to light and therefore from its deleterious effects. Colloidal clay protects viruses from inactivation in natural seawater (Bitton and Mitchell, 1974).

Low temperatures always favor survival of viruses; warm temperatures are destructive. It is also possible that viruses aggregated or adsorbed may experience some heat-sparing, although rotaviruses in sludges become more sensitive to heat, perhaps because of ionic detergents present in sludge (Ward and Ashley, 1980).

Chemical Factors. The pH of the virus environment affects the configuration of the virus capsid and may affect reactions of capsid proteins with chemicals in the water environment. An altered configuration of the capsid may also facilitate entry of noxious moieties into the virion. The pH may also have indirect effects on virus survival; it may facilitate adsorption or elution of the virus particle onto or from solids, and although pH alteration is unlikely to effect aggregation of virions in a dilute suspension, pH alteration may bring about deaggregation.

Heavy metals may be virucidal. Moreover, many cations and anions may affect virus stability in subtle, little-understood ways. Chloride ion, for example, greatly facilitates the virucidal activity of hypochlorite ion by some unknown mechanism. Organic molecules also may be protective or destructive. Some organics may protectively coat the virus particle. Others, such as detergents, may be virucidal under some conditions (Ward and Ashley, 1980).

Biological Factors. Biological inactivation is an important factor in the survival of the virus in the water environment. Viruses, for example, survive longer in autoclaved seawater than in natural seawater indicating that biological flora, or products they produce, are antiviral. Shuval et al. (1971) showed that natural seawater contains a MAVA that could be removed or destroyed by heating, filtration or treatment with ether. Similar agents apparently occur in freshwater (Herrmann et al., 1974). A number of other studies support these findings. Several bacteria have been recovered that may account for the antiviral activity of the waters.

Table III-7 shows the survival of poliovirus 1 seeded into freshwater and into natural seawater (Block, 1983).

Recovery of Viruses from Surface Waters. Although we can measure the numbers of viruses discharged by sewage treatment plants and determine laboratory survival times for viruses in various waters under various conditions, it is difficult to extrapolate such data to estimates of the numbers of viruses in downstream waters. However, over the past 2 decades, technology for recovering viruses from surface waters has improved considerably, and measurements of the numbers of viruses recoverable have been made on many surface waters throughout the world.

Table III-8 shows the results of studies on the recovery of viruses from surface waters collected from Europe and from the United States (Block, 1983). More than 600 infective units of virus/ℓ were recovered from one river and almost 200 and 300 infective units/ℓ were collected from two other rivers.

In a longitudinal study in the United States along the Mississippi and Missouri Rivers, numerous recoveries of viruses were made from 380 ℓ (100-gallon) samples (Table III-9) (Berg et al., 1971; Berg, 1978b). The waters were sampled over courses of hundreds of miles and over a period of more than a year. In 4 of the 18 samples from which viruses were recovered, 400 or fewer fecal coliforms/100 mℓ were recovered. These waters thus met recreational water standards for indicator bacteria (Quality Criteria for Water, 1977). In nine instances, viruses were recovered at or near water intakes. The fecal coliform-to-virus ratios in samples collected near water intakes or at water intakes were lower than the fecal coliformto-virus ratios in samples collected at other locations, indicating that the fecal coliform indicator bacteria died off faster than the viruses because the

TABLE III-7

Survival of Seeded Poliovirus 1 in Freshwater and in Seawater*

Type of Water	Initial Virus Concentration	Temperature (°C)	Time for 99% Inactivation (days)
Freshwater	10^6 PFU	19-25	15
	10^4 PFU	23-27	3
	10^4 PFU	16-23	6
	10^4 PFU	20	6-8
	10^6 PFU	18-20	25
Seawater	$10^{5.8}$ $TCID_{50}$	18-20	2-3
	10^5 PFU	25	4-5
	10^5-10^6 PFU	15	7
	$6x10^2$ PFU	24	3-4
	$5.2x10^3$ PFU	24	10
	10^5 DCP_{50}	18	23
	10^3-10^4 PFU	22	3-12
	10^5 PFU	18-20	3-4
	10^4-10^6 PFU	24	1-4
	$2x10^4$ PFU	20	4-6
	10^5-10^6 PFU	30-33	1-2

*Source: Abstracted and revised from Block (1983)

TABLE III-8

Recoveries of Viruses from Surface Waters*

Surface Water	Country	Maximum No. of Viruses Recovered (PFU or MPJCU/ℓ)
Missouri River	United States	0.1
Seine River	France	191
Thames River	England	22
Lee River	England	12
Rhine River	France	283
Moselle River	France	0.2
Tanana River	United States	0.06
Rio Besos	Spain	55
Rio Lhobregat	Spain	55
Lake Ronkonkoma	United States	1.4
Wear River	England	6
Avon and Sowe Rivers	England	620

*Source: Abstracted and revised from Block (1983)

TABLE III-9

Recoveries of Viruses Along the Mississippi and Missouri Rivers[a]

River	Test No.	Viruses (PFU/380 ℓ)	Fecal Coliforms (CFU/100 mℓ)
Mississippi	1	24	19,600
	2	76	300,000
	3	32	92,000
	4	10	7,800
	5[b]	30	520
	6	20	1,020
	7[b]	5	1,740
	8[b]	2	1,250
	9[b]	1	1,000
	10[b]	4	8,000
	11[b]	3	140
	12[b]	3	400
Missouri	1	5	5,000
	2	10	40,000
	3	4	10,000
	4[b]	19	137
	5	1	60
	6[b]	3	1,900

[a] Source: Abstracted and revised from Berg et al. (1971b) and Berg (1978b)

[b] Recovered at or near water intakes

the distances from the domestic sewage outfalls (the sources of most of the viruses and fecal coliforms) to the water intakes were much longer than the distances from these outfalls to the other sampling points. Such disparities in dieoff rates would be expected if the viruses were more resistant to the water environment than were the fecal coliforms.

Sampling for viruses is expensive and usually no more than one sample is collected at any one place at any one time. However, Block et al. (1978) and Block (1983) took multiple samples at <2-minute intervals from single sites on the Moselle, Rhine and Seine Rivers and found large variations in the numbers of viruses recovered (Table III-10). On the Moselle River, recoveries of >200 PFU/ℓ of river water were made twice over those short intervals, but no viruses were recovered from three other samples taken within the same time frame. From a fifth sample, only 22 PFU/ℓ were recovered. Similar data, though less dramatic, were obtained with sampling on the Rhine and Seine Rivers. These data clearly point up the need to give careful thought to sampling procedures and to the interpretation of negative results.

Viruses in Groundwaters

About 70% of the population of the United States resides in sewered communities. According to Keeley and Kerr (1977), the remaining population supplies ~3.5 billion gallons of sewage daily to the subsurface environment through onsite disposal in septic tanks and cesspools. Federal law intended to eliminate pollutant discharge into rivers and streams by 1985 encourages land disposal and filtration of marginally treated sewage through soil as part of the treatment train. Across this nation, such treatment, widely

TABLE III-10

Numbers of Enteric Viruses in Samples of River Waters Collected at Less Than 2-Minute Intervals*

No. of Consecutive Samples	River (PFU/ℓ)		
	Moselle	Rhine	Seine
1	0	56	1
2	0	0	0
3	210	59	0
4	0	62	0.2
5	22	0	0.1
6	283	ND	ND

*Source: Revised from Block et al. (1978) and Block (1983)

ND = Not done

practiced, adds another 8.2 billion gallons of sewage daily to the subsurface environment (Wellings, 1982):

> "Those soils which score the highest on percolation tests required for septic tank drainfields are the same soils which allow rapid passage of the virus-laden waters. If virus adsorption onto soil particles is to occur, particularly in sandy soils, percolation must be slow enough to permit virus adsorption. The same dichotomy will be operable at sewage effluent land disposal sites."

Mack et al. (1972) recovered a poliovirus from a contaminated well. In subsequent years, a number of recoveries of viruses were made from groundwaters. Wellings et al. (1975, 1977) recovered viruses from groundwaters at effluent land disposal sites. Viruses have also been recovered 3 and 46 m down gradient from sewage effluent recharge basins (Vaughn et al., 1978) and 18 m from a sludge disposal site (Wellings et al., 1977). Viruses were recovered from one well 30.5 m deep. At one site, 14, 67 and 78 PFU of viruses were recovered from 190 ℓ samples of groundwater following heavy rains that may have eluted viruses that had been adsorbed to soil particles (Wellings et al., 1974). Only one or two PFU of viruses had been recovered from 190 ℓ samples of groundwater before the heavy rains. Virus contamination of groundwater probably does not occur continuously, but likely occurs sporadically when pH or other soil factors are altered (Wellings, 1982). Factors other than pH that affect the adsorption and elution and survival of viruses filtered through soil include the nature of the soil (composition and configuration), the presence and nature of cations and other ions, the presence and nature of organics, and the biological activity in soil. Wellings (1982) commented that infrequency of monitoring may be the reason viruses are not found more frequently in groundwaters. She noted that in a study done over a 24-hour period, counts of 67, 14 and 0 PFU resulted, suggesting that a single sample taken during the period could have yielded negative and therefore misleading data.

Viruses in Drinking Waters

Although the frequent recoveries of viruses at water intakes would seem to presage a problem in drinking water, it has been argued that disinfection, especially after complete treatment (coagulation, flocculation and filtration) of water destined for drinking, should obviate any viral hazard in finished waters. Unfortunately, this otherwise reasonable assessment does not allow for the occasional breakdowns occuring in treatment systems, and it does not take into account the protection of viruses by the particulates, fecal debris or cellular detritis, or even the pipe slime in which viruses may be encased. Scarpino (1984) and Hoff (1978) showed that viruses encased in cells are more difficult to inactivate with chlorine than viruses that are not protected and thereby accessable to direct attack by the disinfectant. Chromogenic bacteria recovered from a drinking water that contained ≥2 mg/ℓ of hypochlorous acid were readily inactivated by much lower levels of hypochlorous acid when the organisms were grown in culture and were not encased in protective material (Berg et al., unpublished data).

Table III-11 shows 23 recorded recoveries of viruses from drinking water supplies (AWWA Committee on Viruses in Drinking Water, 1979; Melnick and Gerba, 1982; Berg, 1984). The recoveries date back to 1960 when gauze pads, relatively inefficient for concentrating viruses, were still commonly used. Even with so inefficient a concentration procedure, a large number of virus recoveries were made (Coin, 1966), suggesting that large numbers of viruses

TABLE III-11

Reported Recoveries of Human Viruses from Treated Drinking Waters[a]

Virus Type Recovered	No. Samples Positive/ No. Samples Collected	Water Treatment	Location and Date	Reference
Reo (?)	1/464	complete	South Africa (NR)	Grabow and Isaacson, 1978
Nonpolio, entero	NR	complete[b]	Italy (NR)	Petrilli et al., 1974
Polio, other enterics	43/553	disinfection	France 1960-1962	Coin et al., 1966
Reo, entero	2/25	complete[c]	France 1961-1963	Foliguet et al., 1976
Coxsackie A	2/65	complete	Romania 1962-1971	Nestor and Costin, 1976
Coxsackie A and B, Echo, polio	9/64	complete	Russia 1966-1971	Rabyshko, 1974
Reo, echo, polio	7/64	complete	Massachusetts 1969-1971	Fri and McDermott, 1972
Reo, entero	2/NR	complete[d]	South Africa 1971	Nupen and Stander, 1973
Polio	1/7	complete	Massachusetts 1972-1973	Clarke et al., 1975
Polio, coxsackie A, untyped strains	8/220	complete	Romania 1972-1977	Nestor et al., 1978
Echo	1/10	disinfection	Florida 1975	Wellings et al., 1975
Polio	4/12	complete	Virginia 1975	Hoehn et al., 1977
Polio	(6.7%)	disinfection	Canada, 1975-1976	Sekla et al., 1980
?	1/1	none	United Kingdom 1975-1976	Morris, 1981
Polio	1/42	complete	Virginia 1976	Berry and Shaffer, 1978
NR	19/74	disinfection	India 1978	Rao et al., 1978
Polio, echo	12/18	disinfection	Israel 1978	Marzouk et al., 1980
Coxsackie B	13/31	complete	Mexico 1978	Deetz et al., 1984
Rotavirus	13/31	complete	Mexico 1978	Deetz et al., 1984
Polio	31/31	complete	Canada 1978-1979	Payment, 1981
Polio, coxsackie B, echo	90/553	mostly disinfection	United Kingdom 1979-1982	Tyler, 1983
Hepatitis A, coxsackie B	3/6	disinfection	Texas 1980	Hejkal et al., 1982
Polio, coxsackie B	11/155	complete	Canada 1983	Payment, 1984

---

[a] Source: Modifed from American Water Works Association Committee on Viruses in Drinking Water (1979), Melnick and Gerba (1982) and Berg (1984)

[b] An experimental plant that used complete treatment of a heavily polluted source water; finished product was not consumed.

[c] Complete treatment in general was coagulation-sedimentation, rapid filtration and disinfection.

[d] Further chlorination received before distribution; no viruses recovered after final treatment.

NR = Not reported

were present in the raw waters and that many viruses survived disinfection. Coin's studies were done from 1960-1962 on the water supply of Paris, France at a time when little treatment other than some settling and marginal chlorination (followed by dechlorination) was applied to the raw river source water (Hannoun, 1965). Most of the supplies listed in Table III-11 that yielded viruses had undergone complete treatment, although it is not always clear what constituted complete treatment. Efforts by the U.S. EPA to obtain details on treatment in some of these plants were not successful (Akin, 1977).

In some of the studies listed in Table III-11, viruses were recovered from drinking waters with measurable chlorine present. In an outbreak of gastroenteritis in Georgetown, TX in 1980 that involved 8000 of the town's 10,000 people, coxsackievirus B3 was recovered from drinking water that contained 0.8 mg of total chlorine/ℓ (Hejkal et al., 1982). In other studies in Mexico, a group B coxsackievirus and a rotavirus were recovered from tap waters that contained chlorine (Goyal, 1983; Smith and Gerba, 1981). In the Georgetown epidemic, an outbreak of hepatitis A, traced to the drinking water, followed the sentinel epidemic of gastroenteritis. Hepatitis A antigen was recovered (identified by radioimmunoassay) from the community's sewage and from the wells that supplied the city's drinking water. This is the first apparent recovery of hepatitis A antigen early in an outbreak (Melnick and Gerba, 1982). The hepatitis outbreak peaked 1 month after the antigen was detected in the water.

In two of the better documented studies reported, viruses were recovered from extensively treated reclaimed wastewater (Nupen and Stander, 1973; Grabow and Isaacson, 1978).

Although the reported occurrences of viruses in drinking waters may have been the result of deficiencies in treatment or cross connections, recoveries of viruses were made from waters that met indicator bacteria and other standards. Furthermore, the recoveries were made with methods utilizing concentration procedures whose average efficiencies of recovery did not exceed 20%. Moreover, the cell culture systems used to recover viruses from concentrates were capable of detecting only ~50% of the known enteric viruses occurring in water. Hepatitis A virus, rotaviruses, Norwalk and Norwalk-like viruses, other small round viruses, and the fastidious enteric adenoviruses could not have been detected in most or all of these studies (Melnick and Gerba, 1982). One must presume that as virus recovery methods become more efficient, reported recoveries of viruses from drinking waters will increase in number.

Summary

Although many enteric viruses are not readily detected in sewage, because good methods for detecting them there are not yet available, all enteric viruses probably occur in sewage. Moreover, only one cell culture system is usually used in studies designed to detect viruses in sewage and other environmental waters, and that cell culture system is usually sensitive primarily to enteroviruses. Many other enteroviruses and most other enteric viruses are not detected in such cell culture systems. Viruses known to produce waterborne epidemics, such as Hepatitis A virus, the

Norwalk and Norwalk-like viruses, and the rotaviruses are not detected in those studies. Detection of these known waterborne agents requires newly developed, specialized techniques most of which are not yet useful for studies in environmental waters. In most studies over the years, some of the most important viruses in the water environment have thus been missed.

In warm areas of the world where sanitation is poor and water availability is limited, quantities of viruses in wastewater exceeding 250,000/ℓ have been reported. In the United States, however, wastewaters usually contain 25-250 viruses/ℓ. The viruses found in sewage, are the viruses circulating in the community that produces the sewage. Normally, in temperate climates, most enteroviruses predominate in the community and in the sewage during the warmer months of the year and hepatitis A virus, rotaviruses, and some adenoviruses predominate in the community and in the sewage during the colder months. The patterns in other environmental waters appear to reflect the patterns that occur in sewage.

Viruses survive for long periods in fresh waters and survival is often prolonged for viruses adsorbed to solids. Some viruses enter waterways adsorbed to fecal or other solids. Some viruses become adsorbed in the waterway. Adsorbed viruses often settle to the bottom only to be churned up subsequently by heavy rains or other surges, and transported downstream to recreational areas, water intakes and estuarine shellfish beds. Viruses have been recovered from many such sites. Groundwaters, impacted by sewages and sludges discharged to the land, may be contaminated with viruses.

Numerous reports are now available concerning the recovery of viruses from drinking waters that have received treatment, often complete treatment, including disinfection.

IV. HEALTH EFFECTS IN ANIMALS

Diseases of Animals Produced by Enteric Viruses of Humans

Although some enteric viruses of humans were first detected following inoculation of the viruses into animals and the consequent diseases that resulted in those animals, many enteric viruses of man do not infect any known animals. Moreover, most enteric viruses of man naturally infect man alone; however, some similar enteric viruses are native to various animal species and do not infect man.

Enteroviruses.

Poliovirus --

Species Susceptible. Bodian et al. (1949) reported the existence of three distinct antigenic types of polioviruses that can be distinguished by neutralization tests and by other serological tests. For the polioviruses, man is the only known natural host. Chimpanzees and monkeys tested in captivity may have antibodies to polioviruses, but these antibodies are probably acquired in captivity. Infections with the polioviruses can be induced in old world monkeys and in chimpanzees by the oral, intraspinal and intracerebral routes. Such infections may be produced by freshly isolated strains or by laboratory propagated strains of viruses. Nonprimates are not equally susceptible. However, serially passaged strains of poliovirus have been adapted to mice (Koprowski et al., 1947) and cotton rats (Armstrong, 1939), and strains of poliovirus have been adapted to chick embryos and suckling hamsters (Lennette and Schmidt, 1979).

Although it was initially believed that all polioviruses were obligatorily neurotropic, it is now known that these viruses multiply readily and produce cytopathic changes in many extraneural primate cells. Such cells are used widely to recover, identify and study polioviruses. In earlier years when primates were the only known host for these viruses, Albert Sabin attempted to grow poliovirus in non-neural cells in culture and failed; some years later a successful Nobel prize-winning effort by others achieved this same thing. Upon investigating his own failure subsequent to the successful effort, Sabin found that the poliovirus strain he had randomly chosen to use was a rare obligatorily neurotropic strain that would not grow in non-neural cells (Sabin, 1955). Subsequently, Sabin developed the live virus poliovaccine, which is so extensively used throughout the world today.

Pathogenesis. In chimpanzees, ingested polioviruses multiply in the oropharyngeal mucosa and in the intestinal mucosa. Multiplication of the virus occurs in the tonsils and in the Peyer's patches of the ileum (Bodian, 1955). Viral concentrations as high as 10^7 or 10^8 g of $TCID_{50}$ may accumulate in the tissue. From the primary site of multiplication, the viruses drain into deep cervical and mesenteric lymph nodes and from the nodes into the blood, producing a transient viremia that transports the viruses to the brown fat and to the viscera. Polioviruses may replicate at these sites and feed the blood stream producing a persistent viremia (Bodian, 1956). Usually, however, poliovirus infections do not proceed beyond the lymphatic stage and clinical disease does not occur. When a persistent viremia occurs, spread to the central nervous system through capillary walls may follow and, if sufficient damage occurs in the spinal cord, paralysis and even death may follow (Sabin, 1956). Paralysis, when it occurs, usually results from lesions in the anterior horn cells of the spinal cord. Similar lesions may occur in the medulla and brain stem (bulbar poliomyelitis) and in the cortex (encephalitic poliomyelitis). Bulbar poliomyelitis often results in respiratory or cardiac failure and is thus often fatal. Paralysis in spinal or encephalitic poliomyelitis may resolve partially or totally. When it occurs, paralysis is complete within a few days of onset. Resolution of the paralysis, when it occurs, results from compensatory hypertrophy of muscles that have not lost innervation and from the recovery of nerves that, although never infected, may have become edematous and contributed to the paralysis (Howe and Wilson, 1959).

Coxsackievirus Group A --

Species Susceptible. Newborn mice inoculated intracerebrally, intraperitoneally or subcutaneously with Group A coxsackieviruses suffer a diffuse myositis that produces a total flaccid paralysis. No other lesions occur (Gifford and Dalldorf, 1951).

Intracerebral inoculation of rhesus monkeys with coxsackieviruses A7 or A9 may produce poliomyelitis-like lesions in the CNS and a flaccid paralysis similar to that produced by polioviruses (Dalldorf, 1957).

Many group A coxsackieviruses multiply and produce cytopathic changes in primate cells. Other group A coxsackieviruses recovered from clinical specimens can be studied only in young mice. Although newborn mice (1-2 days old) are the animal host of choice for group A coxsackieviruses, these viruses multiply in 4- to 5-day-old mice also.

Pathogenesis. Group A coxsackieviruses produce severe inflammation and necrosis of voluntary muscle fibers in newborn mice (Gifford and Dalldorf, 1951). A flaccid paralysis results. When muscle necrosis is sufficiently severe, large quantities of myoglobulins are liberated, thus causing renal lesions.

Rhesus monkeys infected intracerebrally with coxsackieviruses A7 or A9 may suffer extensive degeneration of ganglionic cells of the CNS followed by flaccid paralysis (Dalldorf, 1957).

Coxsackievirus Group B --

Species Susceptible. Group B coxsackieviruses inoculated intracerebrally, intraperitoneally or subcutaneously into newborn mice ≤1-day-old produce tremors, spasticity and spastic paralysis (Gifford and Dalldorf, 1951). Pancreatitis may also develop (Pappenheimer et al., 1951). Adult mice may be rendered susceptible by cortisone administration, X-irradiation, cold or severe malnutrition.

Group B coxsackieviruses grow well and produce cytopathic changes in many cell culture systems (Girardi et al., 1957).

Pathogenesis. Group B coxsackieviruses produce focal areas of degeneration in the brain, focal necrosis in skeletal muscles, and inflammation in the pancreas, dorsal fat pads and sometimes in the myocardium of mice ≤1-day-old. Necrosis of the brown fat pads is the most salient pathologic lesion in newborn mice along with encephalomalacia, myocarditis, pancreatitis and hepatitis. Adult mice develop pancreatitis primarily.

Necrosis of the myocardium may also occur, however (Gifford and Dalldorf, 1951). Infections in newborns are usually fatal; infections in adults are usually not fatal except in pregnant or cortisone-treated mice (Kilbourne et al., 1956).

Echovirus --

Species Susceptible. At least 14 echoviruses (1-4, 6-9, 13, 14, 16-18 and 20) inoculated intraspinally or intracerebrally into rhesus or cynomolgus monkeys produce infection. Some strains of echoviruses 6 and 9 infect newborn mice. Echoviruses grow well and produce cytopathic changes *in vitro* in many cells of primate origin. Cultures of rhesus, cynomolgus and African green monkey kidney cells are most frequently used for propagation of these viruses (Drouhet, 1955).

Pathogenesis. Echoviruses 1-4, 6-9, 13, 14, 16-18 and 20 inoculated intraspinally or intracerebrally into rhesus or cynomolgus monkeys produce viremia, neuronal lesions and meningitis sometimes associated with muscle weakness. The strains of echoviruses 6 and 9 that infect newborn mice produce lesions in animals similar to those produced by Group B and Group A coxsackieviruses, respectively.

Enteroviruses 68-72 -- Because of overlapping species susceptibilities, new nonpoliovirus enteroviruses are no longer named echoviruses or coxsackieviruses but rather enteroviruses beginning with enterovirus 68. Included in this new classification is enterovirus 72, hepatitis A virus.

Species Susceptible. Enterovirus 72 infects only man in the natural environment, but chimpanzees associated with man sometimes acquire the disease. Chimpanzees are also experimental hosts for enterovirus 72 (Dienhardt and Gust, 1982). The only other available experimental hosts for this virus are marmosets and some species of monkeys (Lemon et al., 1982).

Enterovirus 72 infects some cells in culture (Provost et al., 1982) but does not cause CPE or death in most of them (Gerba, 1984). The virus may be detected in these cells by flourescent antibody, RIA and radioimmunofocus assay techniques (Gerba, 1984). The HM 175 strain of enterovirus 72 has been adapted to cell cultures and can be assayed by immunoflourescence and by other methods.

Pathogenesis. In the chimpanzee infected orally, enterovirus 72 is believed to multiply in the GI tract and may involve the mesenteric lymph nodes, eventually resulting in a viremia. The virus may spread to the kidneys, spleen and liver. When the liver is infected, jaundice (icterus) may result. Virions may be detected in the feces, urine and blood during the preicteric and early icteric phases of the disease. The virus reaches its highest concentration in the days just before the onset of jaundice. Shedding of virus ceases shortly after onset of jaundice. When found in the feces, virions may also be found in the liver and bile.

Other Viral Genera.

Adenovirus --

Species Susceptible. Intravenous inoculation of many adenoviruses into rabbits and intranasal instillation of many of these viruses into guinea pigs, dogs, hamsters and piglets produces inapparent infections. Adenovirus 5 may persist in the spleen of rabbits for a period of 6 months and manifest its presence subsequently in vitro in explant cultures. Adenoviruses 3, 7, 11, 14, 16 and 21 (adenovirus subgroup I) and adenoviruses 12, 18 and 31 (adenovirus subgroup IV), when inoculated in large quantities in newborn rats, mice and hamsters may produce tumors.

Adenoviruses grow in many different mammalian cell cultures including HeLa cells and the primary human embryonic kidney. Adenoviruses 40 and 41 are fastidious but replicate in Graham 293 cells (Takiff et al., 1981), and to some extent in other lines as well (deJong et al., 1983).

Pathogenesis. Adenoviruses of subgroups I and IV may produce tumors in newborn rats, mice and hamsters when the viruses are inoculated in large quantities.

Reovirus --

Species Susceptible. The reoviruses are ubiquitous in nature. Most species in nature exhibit serum inhibitors, presumably antibodies to these viruses. Monkeys, cattle, mice and many other species are infected with reoviruses in their natural environment. Reoviruses grow in many primary and continuous primate cell lines and produce cytopathic changes in infected cultures.

Pathogenesis. Newborn mice infected experimentally often die. Reovirus 3 some times produces a chronic illness similar to runting in newborn mice. Encephalitis, pneumonia and hepatitis have been produced in experimental animals with these viruses.

Rotavirus --

Species Susceptible. Human rotaviruses may infect piglets, newborn rhesus monkeys and gnotobiotic newborn calves. The virus can be detected in the duodenal mucosa and in the feces of infected animals. Rotaviruses infect a variety of species (Holmes, 1982). Rotaviruses that naturally infect calves, offal, mice and monkeys are immunologically similar or identical to the human rotaviruses but their RNA contents and their proteins differ.

Human rotaviruses can be propagated serially in human fetal intestine organ cultures, in primary embryonic human intestine, and in pig intestine or kidney cells. Only occasional cells are infected, however, and the virus must be demonstrated by immune electron microscopy and by immunoassay techniques. The WA strain of type 2 rotavirus (human) has been adapted to African green monkey kidney cells and can be plaqued. Rotaviruses have recently been recovered from human stools using primary African Green monkey kidney cells in which the rotaviruses produce no CPE but can be detected by ELISA. These rotaviruses can be plaqued in MA 104 cells after several passages in primary monkey kidney cells (Ward, 1984).

Pathogenesis. Experimentally, human rotaviruses replicate and produce lesions in the mucosal and submucosal cells of the small intestines of piglets and rhesus monkeys, but not in the large intestines or in any other organ.

Norwalk, Norwalk-like and Other Small Round Viruses --

Species Susceptible. Norwalk, Norwalk-like (Gerba et al., 1984), and the other small round viruses have not yet been shown to infect animals or cell cultures.

Pathogenesis. Pertinent data regarding the pathogenesis of these viruses could not be located in the available literature.

Minimal Infective Dose of Human Viruses in Animal Hosts

The term "minimal infective dose" is defined and explained in Chapter V.

Relatively few data exist on the minimal infective dose for animal viruses in their natural hosts. Even fewer such data exist for human viruses in animal hosts. However, the data available indicate that small quantities of viruses, as measured in cell cultures or in other host systems, can infect animal host species.

Available data demonstrate the capability of small quantities of viruses as measured in one host system to infect another host system. Tables IV-1 to IV-3 show that the smallest quantity of viruses demonstrable in one host system can be sufficient to infect another host system.

TABLE IV-1

Infective Dose by the Respiratory Route of Coxsackievirus B4 for Newborn Mice*

Dose by the Respiratory Route/ Intraperitoneal LD_{50} for Newborn Mice	Percent Mortality
1.3	30
13	30
130	50
1,300	100
13,000	100

*Source: Abstracted and revised from Suptel (1963)

TABLE IV-2

Infective Dose by the Intranasal Route of *Myxovirus parainfluenza* 3 for Hamsters[a]

No. of $TCID_{50}$ Inoculated[b]	No. of Hamsters Infected/ No. of Hamsters Inoculated
0.03	0/3
0.3	1/3
3.2	2/3
32	3/3
320	3/3

[a] Source: Revised from Craighead et al. (1960)

[b] $TCID_{50}$ determined in rhesus kidney cell cultures

TABLE IV-3

Infective Dose by Inhalation of Rift Valley Fever Virus for Hamsters*

Dose Inhaled/ Intraperitoneal LD_{50} for Mice	Hamsters Dead/ Hamsters Inoculated
0.2	6/24
0.7	14/24
2	20/24
14	23/24

*Source: Revised from Miller et al. (1963)

Table IV-1 shows that 1.3 i.p. LD_{50} of newborn mice killed 30% of newborn mice inoculated by aerosol inhalation (Suptel, 1963). Since some of the surviving mice may have been infected, it is possible that the ID_{50} was <1.3 of i.p. LD_{50} (Plotkin and Katz, 1967).

Table IV-2 shows that 0.3 of Rhesus kidney $TCID_{50}$ (50% tissue culture infective doses) of parainfluenza 3 virus infected one of three hamsters inoculated intranasally (Craighead et al., 1960). These data indicate that one $TCID_{50}$ of the inoculum was equal to one ~50% hamster-infecting dose. Cook and Chanock (1963) showed subsequently that one rhesus kidney cell culture $TCID_{50}$ of parainfluenza virus types 1, 2 and 3 given intranasally could infect the lungs of hamsters.

Table IV-3 indicates that, administered by the aerosol route, <0.7 of mouse i.p. LD_{50} of Rift Valley fever virus constituted one LD_{50} in hamsters (Miller et al., 1963). Miller et al. (1963) also showed that six mice i.p. LD_{50} infected all monkeys exposed to an aerosol of the Rift Valley fever virus.

Mice and guinea pigs have been infected with aerosols containing one guinea pig heart cells $TCID_{50}$ of attenuated Venezuelan encephalitis virus (Kuehne et al., 1962).

V. HEALTH EFFECTS IN HUMANS

Diseases of Man Produced by Enteric Viruses of Man

Enteroviruses. The enteroviruses comprise a number of different groups the classification of which, based on pathology and host range, has blurred in recent years. These enteroviruses include the polioviruses, group A coxsackieviruses, group B coxsackieviruses, echoviruses, and other enteroviruses. The other enteroviruses are all new classified enteroviruses that are not polioviruses. Currently, these enteroviruses number from 68 to 72 (Matthews, 1982).

Poliovirus -- The polioviruses comprise three distinct immunological types designated polioviruses 1 to 3. All may produce paralytic disease in man, but within each type there are strains covering a wide range of neurovirulence. As measured by the results of intraspinal or intracerebral inoculation of virus into monkeys or chimpanzees, some strains of poliovirus produce devastating destruction of nervous tissue, paralysis and death. At the other end of the spectrum are attenuated strains that appear incapable of producing any damage at all. Many strains of polioviruses, should they reach the CNS, are capable of producing damage of varying degrees between the extremes just described (Lennette and Schmidt, 1979).

Until the beginning of this century, poliomyelitis was primarily a disease of infants and young children. Until the middle of this century, the disease was largely known as infantile paralyses. In the days when the disease in the United States was primarily an infantile disease (in less developed nations today the disease is often still an infantile disease), almost all children were infected with all types of the virus by the age of

5, the disease was usually subclinical (inapparent), and paralysis was rare. As sanitation improved in countries such as the United States, and the rate of transmission fell, infections began to appear later in life. With the change in the age incidence of infection, greater numbers of paralytic cases appeared. It became apparent that, as with certain other viral diseases, the severity of the disease increased with increasing age. By the mid-1950s, when the first poliovaccines became available, many people lived their entire lives without having been infected with all three types of poliovirus. But, few if any people had not been infected with at least one or two types of the virus. Even with the increased risk of severe disease in adulthood, the incidence of such serious consequences was still low. However, in crowded areas where sanitation was poor, however, even in the United States, infection with polioviruses still tended to be more frequent at younger ages (Lennette and Schmidt, 1979).

Mode of Transmission. The major mode of transmission for polioviruses is the fecal-oral route, usually accomplished by direct contact (Lennette and Schmidt, 1979).

Pathogenesis. After ingestion, the poliovirus multiplies in the oropharyngeal mucosa and in the intestinal mucosa. The virus invades the tonsils and the Peyer's patches of the ileum, both of which are lymphoid tissues, and multiplies extensively there. One million to 10 million PFU of poliovirus (as measured in cell cultures) may accumulate per gram of tissue. The viruses drain into the deep cervical and mesenteric lymph nodes and from there into the bloodstream setting up a transient viremia that transports the virus to all organs of the body. The virus multiplies in the brown fat (probably in other tissues also) and continuously feeds persistent viremia. Consequent to the persistent viremia, the poliovirus may penetrate through capillaries into the CNS where sufficient progressive damage may produce weakness, flaccid paralysis and death. Most infections with polioviruses do not progress beyond the transient viremia stage. However, the virus can also be transported along nerve fibers to the brain and spinal cord as is the case in provocation (Belsche, 1984).

The total incidence of paralytic disease is ~1-2% of cases. Transient viremia may be accompanied by symptoms including fever and constitutional and GI signs. In cases of severe disease, these symptoms are followed 1-3 days later by headache, fever, stiffening of muscles, signs of meningeal irritation (aseptic meningitis), and sometimes paralysis. Paralysis may result from lesions in the anterior horn cells of the spinal cord (spinal poliomyelitis), lesions in the brain stem and medulla (bulbar poliomyelitis), or lesions in the motor cortex (encephalitic poliomyelitis). The bulbar form is often fatal, because it may bring about respiratory or cardiac failure. When paralysis occurs, it becomes maximal within a few days after onset. Some degree of recovery may eventually occur, because although regeneration of nervous tissue does not occur, some compensatory hypertrophy of muscles does, and some damaged neurons that may add to the paralysis may regain function (Belsche, 1984).

Immunity. Antibodies to infecting polioviruses begin to appear about 1 week after infection and persist for life protecting against paralytic disease but not against reinfection. An individual infected with a poliovirus by the oral route is refractory to reinfection by that route for some weeks or months (Belsche, 1984).

Coxsackievirus Group A -- The coxsackieviruses, both groups A and B, were first isolated during a search for polioviruses in Coxsackie, New York, hence the name given to the viruses. Twenty-three immunologically distinct types of group A coxsackieviruses have been classifed (Belsche, 1984).

Mode of Transmission. Most group A coxsackieviruses are transmitted primarily by the fecal-oral route. Some may be transmitted by the respiratory route.

Pathogenesis. Group A coxsackieviruses produce a wide spectrum of illnesses, usually mild. Some of these viruses, however, have not been associated with any diseases. Among those group A coxsackieviruses associated with diseases, some individual viruses produce a variety of diseases. Most of the illnesses produced by group A coxsackieviruses are not distinctive and are also produced by a number of other viruses. However, one disease, herpangina, is uniquely produced only by several types of A coxsackieviruses (Table V-1). Herpangina is a disease, acute in onset, characterized by fever, sore throat, headache, anorexia, dysphagia and occasionally by stiffness in the neck area. Lesions occur in the throat that begin as small papules that soon become circular and ulcerative. The lesions are diagnostic. Table V-1 shows that many group A coxsackieviruses produce aseptic meningitis, respiratory infections, rashes and fevers. Paralysis may be produced by some types, but it is transient.

Immunity. Antibodies specific for the infecting virus type generally appear in the blood within a week after infection is initiated. Maximum titer is usually achieved several weeks after antibodies begin to

TABLE V-1

Association of Clinical Syndromes with Group A Coxsackieviruses*

Syndrome	Coxsackievirus Type
Paralytic disease	2, 7, 9
Aseptic meningitis	2, 4-7, 9, 10, 12, 16
Herpangina	2, 4-6, 8-10, 16, 21
Exanthem (rash), fever	2, 4, 9, 16
Upper respiratory illness	10, 21, 24
Fevers	Most

*Source: Belshe, 1984

appear. Antibodies demonstrated by neutralization tests are long lasting -- sometimes for a lifetime. Immunity to the diseases caused by the particular virus is probably also lifelong.

Coxsackievirus Group B -- Group B coxsackieviruses comprise six distinct immunologic types, which are immunologically distinct from all 23 group A coxsackieviruses. Group B coxsackieviruses are differentiated from group A coxsackieviruses on the basis of the pathologic lesions produced by members of the two groups of viruses in newborn mice (Lennette and Schmidt, 1979).

Mode of Transmission. Most group B coxsackieviruses are transmitted by the fecal-oral route, although some group B coxsackieviruses produce respiratory diseases and may be transmitted by the respiratory route.

Pathogenesis. Like the group A coxsackieviruses, group B coxsackieviruses produce a wide spectrum of diseases (Table V-2). Similar to group A coxsackievirus infections, most group B coxsackievirus infections are mild. Subsequent to infection, these viruses multiply and produce viremia in much the same way that polioviruses do. However, in the newborn, an often fatal myocarditis is produced by coxsackieviruses B2 to B5. In this disease, the myocardium becomes edematous and gross focal necrosis and acute inflammation occur. Focal necrosis and inflammation occur also in the pancreas, liver, adrenals and skeletal muscles. Diffuse meningoencephalitis may also occur. Some group B coxsackieviruses may also cause a mild interstitial focal myocarditis and sometimes a valvulitis in infants and children. Coxsackieviruses B1 to B5 produce pleurodynia (epidemic myalgia

TABLE V-2

Association of Clinical Syndromes with Group B Coxsackieviruses*

Syndrome	Coxsackievirus Type
Paralytic disease	3-5
Aseptic meningitis	1-6
Upper respiratory illness	2-5
Pleurodynia	1-5
Myocarditis in newborns and children	2-5
Pericarditis	1-5
Fevers	1-6
Exanthem	4

*Source: Belshe, 1984

or Bornholm disease), an acute febrile disease with a sudden onset of severe pain in the chest that is aggravated by deep breathing and movement. Muscle tenderness may also occur. Group B coxsackieviruses may also cause paralytic disease simulating poliomyelitis but resolving relatively quickly, respiratory illness, hepatitis, pancreatitis and nephritis. Group B coxsackieviruses may also be responsible for some congenital heart disease. Women who suffer infections with group B coxsackieviruses during the first trimester of pregnancy deliver infants with twice the usual incidence of congenital heart lesions.

Immunity. Within a week after infection with a group B coxsackievirus, antibodies specific to the infecting virus type usually appear in the blood. Within 2 weeks after the first appearance of antibodies, maximum levels of antibodies are reached in the blood. Neutralizing antibodies are long lasting and probably lifelong, as is the immunity to the diseases produced by group B coxsackieviruses.

Echovirus -- Thirty-two immunologically distinct echoviruses have been classified. "Echo" is an acronym for enteric, cytopathic, human, orphan, denoting that these viruses derive from the enteric tracts of humans, produce cytopathic changes in cell cultures, and that they were orphans in search of a disease (the latter reflecting the initial recoveries of these viruses from healthy children). However, over the years most echoviruses have been associated with diseases (Table V-3) (Belshe, 1984; Lennette and Schmidt, 1979).

TABLE V-3

Associations of Clinical Syndromes with Echoviruses*

Syndrome	Echovirus Type
Aseptic meningitis	1-7, 9, 11, 13-16, 18, 19, 30
Paralysis	1, 2, 4, 6, 9, 11, 16, 18, 30
Encephalitis	2-4, 6, 7, 9, 18, 19
Neuronal damage	17, 20-22, 25, 31
Rash	1-7, 9, 14, 16, 18, 19
Enteritis	6, 8, 11, 12, 14, 18-20
Upper respiratory infections	4, 8, 11, 19, 20, 22, 25
Pleurodynia	6
Myocarditis	6, 19

*Source: Belshe, 1984; Lennette and Schmidt, 1979

Mode of Transmission. Most echoviruses are transmitted by the fecal-oral route, although some produce respiratory diseases and probably are transmitted by the respiratory route.

Pathogenesis. Following initial infection of the GI and respiratory tracts, dissemination of echoviruses through viremia to other target organs may occur with consequent fever, rash, and CNS involvement. Echoviruses have been recovered from blood and from cerebrospinal fluid in aseptic meningitis. In rarely fatal cases of aseptic meningitis, cerebral edema and focal destructive and infiltrative lesions have been observed in the CNS. Echoviruses have been associated with occasional paralytic disease, encephalitis, fever and rash, enteritis, upper respiratory infections, pleurodynia and myocarditis (see Table V-3) (Belshe, 1984; Lennette and Schmidt, 1979).

Immunity. Specific neutralizing antibodies to infecting echoviruses begin to appear within a week after infection is initiated. Neutralizing antibodies to echoviruses are long lasting (probably lifelong) immunity to the diseases produced by the infecting virus type is also lifelong.

Enteroviruses 68-72 (Including Hepatitis A Virus) -- All new enteroviruses that are not polioviruses are now classified as enteroviruses. The first enterovirus so classified was enterovirus 68 (Matthews, 1982).

Mode of Transmission. The modes of transmission for enteroviruses 68-72 are the same as those for other enteroviruses. Most transmission is by the fecal-oral route. Respiratory viruses and those producing ophthalmic disease are probably transmitted by the respiratory and ophthalmic routes, respectively (Lennette and Schmidt, 1979; Belshe, 1984).

Pathogenesis. Special importance is attached to enteroviruses 70 and 72. Enterovirus 70 was recovered from many cases of acute hemorrhagic conjunctivitis that occurred in India, Asia, Africa and Europe from 1969-1974. Infection produced sudden swelling, watering, congestion and pain in the eyes. These symptoms were accompanied by subconjuctival hemorrhages. The disease appears to be self-limiting, subsiding rapidly within 1-2 weeks after onset (Belshe, 1984).

Enterovirus 72 is hepatitis A virus. Its incubation period averages about 30 days. Enterovirus 72 may produce an asymptomatic infection, anicteric hepatitis, hepatitis with severe liver inflammation, and death. The disease produced by enterovirus 72 is often debilitating, and recovery is usually slow. Hepatitis A virus usually infects by the fecal-oral route and apparently multiplies in the GI tract. The mesenteric lymph nodes may be involved. Viremia transports the virus to the liver, kidneys and spleen. The virus is present in the feces, blood and urine during the preicteric period and early in the icteric period. The concentration of enterovirus 72 in the feces reaches its highest level just before jaundice occurs. When the virus occurs in the feces, it is usually also found in the liver and in the bile. Antibodies to the virus appear as the concentration of viruses in the feces diminishes and as liver damage begins to appear. The mechanisms responsible for the course and symptoms of hepatitis A-induced disease have not been ascertained, but the absence of a carrier state suggests that the virus plays a direct cytopathic role (Robbins and Cotran, 1979).

Immunity. Following infection, immunity to disease with enterovirus 72, and with enteroviruses 68-71 as well, is probably lifelong. Antibodies to enterovirus 72 occur in the blood serums of 25-30% of all adults in the United States. However, in some developing nations, probably because of poor sanitary practices, 90% of all children age 15 and under have antibodies to hepatitis A virus (Aach, 1976).

Other Viral Genera.

Adenovirus -- The adenoviruses were first recovered from uninoculated human adenoid tissues growing in culture after cytopathic changes began to appear in the cells of the culture following prolonged incubation. At about the same time, similar viruses were recovered from respiratory secretions of military recruits suffering from influenza-like illnesses. The name "adenoviruses" derives from the latently infected tissues that first yielded the viruses. The viruses cause acute respiratory diseases and eye infections. Forty-one immunologically distinct adenoviruses have been classified (Cukor and Blacklow, 1984).

Mode of Transmission. Adenoviruses are more readily recovered from the feces than from any other body excretion, but these viruses are transmitted primarily from person-to-person by respiratory and occular secretions. Swimming pool transmission also occurs and has been implicated in epidemics of conjunctivitis and pharyngoconjunctival fever. Epidemic keratoconjunctivitis, caused by adenovirus 8, has been associated with trauma to the conjunctiva produced by dirt and dust in shipyards and similar environments and has been transmitted also by improperly sterilized ophthalmic instruments (Lennette and Schmidt, 1979; Belshe, 1984).

Pathogenesis. Adenoviruses are responsible for ~5% of the respiratory diseases in the population (Table V-4). Adenoviruses produce inapparent infections and usually self-limiting illnesses of the respiratory tract and eyes. Adenoviruses 3 and 7 have been recovered from fatal pneumonias in military recruits. The lesions produced in the lungs of the fatal cases were those of viral bronchopneumonia, but there were present also many epithelial cells that contained basophilic masses in their nuclei similar to those that appear in cell cultures infected by the same adenovirus types. Similar findings have been reported following the deaths of two infants from viral pneumonia, one of which yielded adenovirus 7a (Boyer et al., 1957; Chaney et al., 1958). Acute hemorrhagic cystitis in children is apparently produced by adenovirus 11.

Subsequent to infection, most adenoviruses multiply in the GI tract. The viruses are readily recovered from the feces of infected individuals. Adenoviruses 1, 2, 5, 6, and occassionally others have been recovered from acute infectious diarrheas, but a distinct link between these viruses and the illnesses has not yet been definitely established. Adenoviruses 40 and 41, however, the so-called enteric adenoviruses, occur in the feces of infants and young children with gastroenteritis in a frequency second only to that of the rotaviruses (Vesikari et al., 1981; Yolken et al., 1982; Brandt et al., 1983; Uhnoo et al., 1983). Enteric adenovirus infections

TABLE V-4

Clinical Syndromes Produced by Adenoviruses*

Syndrome	Adenovirus Type
Pharyngitis and pharyngoconjunctival fever	3, 5, 7, 21
Conjunctivitis	2, 3, 5-7, 9-11
Epidemic keratoconjunctivitis	7, 8, 19
Acute respiratory disease of recruits	3, 4, 7, 11, 14, 21
Pneumonia in infants	7
Acute hemorrhagic cystitis	11
Gastroenteritis	40, 41

*Source: Lennette and Schmidt, 1979; Belshe, 1984 occur mostly in children under 3 years of age (Uhnoo et al., 1983). Infections are characterized primarily by diarrhea with an average 9-day duration. Fever, vomiting and respiratory symptoms also occur. The enteric adenoviruses have been detected primarily by EM techniques, but these viruses are now known to multiply in Graham 293 cells (Takiff et al., 1981) and in other cells as well (deJong et al., 1983).

Viremias do not usually occur in adenovirus infections, and although adenoviruses are not usually widely distributed throughout the body, cases of adenovirus meningitis have been reported (Lennette and Schmidt, 1979; Belshe, 1984).

Adenovirus infections are common in children. Fifty to 80% of all normal tonsils and adenoids removed surgically and cultivated _in vitro_ yield latent adenoviruses, usually types 1, 2 and 5, the types frequently responsible for infections in young children. Latent adenoviruses have also been recovered from mesenteric lymph nodes (Lennette and Schmidt, 1979; Belshe, 1984). Some human adenoviruses produce tumors in animals, but no adenovirus as yet has been associated with tumors in man (Lennette and Schmidt, 1979; Belshe, 1984).

Immunity. Long lasting specific neutralizing antibodies are produced against infecting adenoviruses. Resulting immunity is long lasting and probably lifelong (Lennette and Schmidt, 1979; Belshe, 1984).

Reovirus -- Three antigenically related immunologic types of reoviruses have been classified. The name reovirus itself, derives from an acronym comprising the first letters of the words "respiratory," "enteric" and "orphan." The reoviruses were originally recovered from cases of respiratory and enteric diseases, but these viruses have yet to be proven to be etiologic agents of diseases in man and so still remain orphans in search of a disease (Matthews, 1979). Inapparent infection with reoviruses are common, however; in the United States, 10% of all children under 5 years of age have antibodies to these viruses, and 65% of all young adults as well.

Mode of Transmission. Reoviruses have been recovered from throat washings and from feces. Presumably, these viruses may be transmitted both by the respiratory and the fecal-oral routes. Reoviruses are widely distributed in nature and are commonly found in the feces of many animals (Lennette and Schmidt, 1979; Belshe, 1984).

Pathogenesis. Reoviruses have been recovered from individuals with mild respiratory or GI illnesses and from many healthy persons as well. It is unclear whether reoviruses produce these illnesses. Three deaths have been attributed to infection with reovirus 3. The lesions produced in these fatal incidents were similar to those produced in experimental animals (Lennette and Schmidt, 1979; Belshe, 1984).

Immunity. Neutralizing antibodies are produced against all three types of reoviruses.

Rotavirus -- Rotaviruses are the most important etiological agents worldwide of diarrhea in infants and young children. These viruses were first detected by immune EM. At least four immunologically distinct serotypes of rotaviruses have been identified (Wyatt et al., 1983). The disease is sporadic in occurrence and, in developing countries, a leading cause of death of children from 6-24 months of age. In the Washington, DC area, >90% of the children studied had antibodies to rotaviruses by the age of 3 (Figure V-1) (Kapikian et al., 1978). Rotavirus infections may be encountered in adults, also.

Mode of Transmission. The mode of transmission of rotavirus infections is thought to be largely fecal-oral, although the incidence of the disease is greatest in cold weather, a characteristic more common to respiratory transmission than to fecal-oral transmission. Waterborne transmission has also been reported (Harris et al., 1983).

Pathogenesis. Rotaviruses produce severe diarrhea, fever and vomiting primarily in infants and in children under 2 (50% of the most severe illnesses of infants and young children seen in hospitals). Rotavirus infections occur in adults also (Wenman et al., 1979). Outbreaks of rotavirus-induced traveller's diarrhea have been reported repeatedly (Bolivar et al., 1978; Echeverria et al., 1981; Ryder et al., 1981; Sheridan et al., 1981), and there have been reports of rotavirus disease outbreaks in a cardiology ward (Holzel et al., 1980) and in a nursing home (Halvorsrud and Oestavik, 1980). Rotaviruses multiply in the intestinal mucosa. Excretion of viruses reaches a maximum during the first 4 days of illness, but excretion may continue for some time after that.

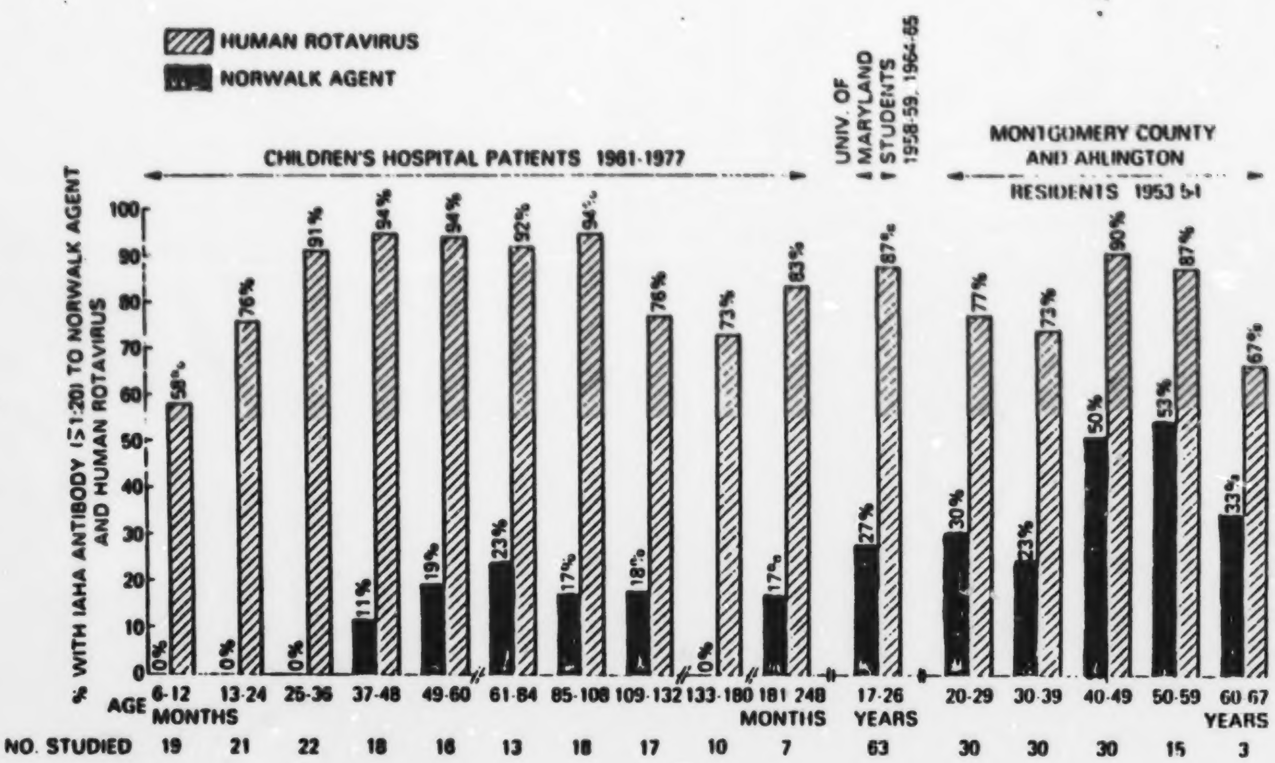

FIGURE V-1

Prevalence of Antibody to Norwalk Virus and to Rotavirus by Immune Adherence Hemagglutination assays (IAHA)

Source: Kapikian et al. (1978)

Human rotaviruses invade the epithelium of the jejunum and primarily affect cells of the villi. All of the small intestines may eventually become involved, although neither cells of the stomach nor of the large intestines seem to be extensively invaded (Davidson et al., 1975). The degree of involvement of the regional lymph nodes and submucosa are unknown but are probably not extensive (Hamilton and Gall, 1982). The enteric bacterial flora seems unaffected in infants infected with rotaviruses (Middleton et al., 1974). Vomiting in rotavirus infection is apparently brought about by infection of the proximal small intestinal mucosa. During the acute diarrheal stage of infection, cells of the villi fail to fully differentiate as they migrate along the villi, resulting in maladsorption of glucose and sodium (Hamilton and Gall, 1982).

Immunity. Antibodies are produced in response to rotavirus infections. The length of time during which immunity persists is not known.

Norwalk and Norwalk-like Viruses -- Epidemic acute gastroenteritis produced by Norwalk and Norwalk-like viruses is a common illness in the United States. Four, and perhaps five, immunologically distinct types of Norwalk and Norwalk-like viruses have been identified. None of these viruses has yet been grown in cell cultures or in laboratory animals. Norwalk and Norwalk-like viruses have been identified in human feces by immune EM and by RIA (Lennette and Schmidt, 1979; Belshe, 1984).

Mode of Transmission. The mode of transmission of Norwalk and Norwalk-like viruses appears to be the fecal-oral route, but some respiratory spread may occur. Norwalk and Norwalk-like virus disease outbreaks are seen in all seasons. Waterborne transmission appears to be a major pathway for epidemic spread (Kaplan et al., 1982).

Pathogenesis. Norwalk and Norwalk-like viruses occur in outbreaks and produce nausea, vomiting, diarrhea, abdominal pain and fever. Infection involves the small bowel primarily. The pathology appears to be similar to that produced by rotavirus infections (Schreiber et al., 1973). Illness usually lasts for 1-2 days and is milder than rotavirus-induced disease. Norwalk and Norwalk-like virus outbreaks are often family and community wide. The incidence of Norwalk and Norwalk-like virus gastroenteritis is highest in school-age children and in adults. The viruses are found in the stools in greatest numbers within 24 hours after clinical onset of the disease. The incubation period is about 48 hours.

Immunity. Norwalk and Norwalk-like virus infections produce antibodies, but antibodies do not appear to confer immunity (Parrino et al., 1977). The epidemiology of Norwalk and Norwalk-like viruses is different from that of rotaviruses (see Figure V-1).

Minimal Infective Dose of Human Viruses in Humans

The concept of minimal infective dose has been reviewed (Plotkin and Katz, 1967; Westwood and Sattar, 1976; Ward and Akin, 1984).

The most often used units of dose in virology are the PFU and the 50% response dose, usually the ID_{50} or LD_{50}. The PFU is the single virus particle or clump of virus particles (viruses often occur naturally in clumps) that produces one plaque. The 50% response dose is that quantity of virus that produces an effect (infection and death in the cases of the ID_{50} and LD_{50}, respectively) in 50% of the animals inoculated.

Although the number of viable virus particles that constitute the infective or lethal dose may be one or more, the number required to produce the initial infection of a cell is one (Beard, 1967; Westwood and Sattar, 1976). Moreover, Plotkin and Katz (1967) and Westwood and Sattar (1976) concluded that one cell culture infective dose is sufficient to infect man. Both reviewed the literature and summarized data supporting that conclusion. Westwood and Sattar (1976) listed nine published reports, in addition to their own data, in which the cell culture infective dose for an animal host or for man was ≤ 1. Ward and Akin (1984), however, noted that the sensitivity of a cell culture system can sometimes be enhanced, and that such enhancement can greatly increase the cell culture infective dose to human infective dose ratio.

Interest in minimal infective dose is a reflection of efforts to determine whether the small number of viruses that might be recovered from a drinking water is sufficient to produce an infection in an individual who consumes that small number of viruses. Since the answer to this question has not yet been determined with epidemiologic techniques, attempts have been made to find the answer by comparing the infectivity of various viruses in the cell culture systems used to recover viruses from water with the infectivity of those viruses in man. The problem with this approach is the absence of a cell culture system that equals the sensitivity of man to all viruses and to all of the strains of viruses that come down the river. One way to enhance the sensitivity of a cell culture system or of an animal host to a strain of virus is to pass the strain serially in the cell culture system or in the animal host. As this strain adapts to its new host it will often lose virulence for its former host. This is an attenuation procedure often used in developing strains of viruses for vaccines; there are many other ways in which host sensitivities to infection with viruses become altered.

A number of studies have been published in which small numbers of viruses, primarily vaccine strains, produced infections in human subjects. Koprowski (Koprowski, 1956; Koprowski et al., 1956) fed poliovirus 1 (SM) in gelatin capsules to adult volunteers and infected 2 of 3 subjects with 2 PFU of the virus (Table V-5). Plotkin and Katz (1967) and Katz and Plotkin (1967) administered attenuated poliovirus 3 (Fox) by nasogastric tube to infants and infected 2 of 3 with 10 $TCID_{50}$ and 3 of 10 with 1 $TCID_{50}$ of the virus (Table V-6). Minor et al. (1981) administered attenuated poliovirus 1 vaccine orally and infected 3 of 6 two-month-old infants with 50 $TCID_{50}$ of the virus (Table V-7). Okuno (1962) administered attenuated measles virus by intranasal spray to children and infected 8 of 35 with 1 $TCID_{50}$ of the virus. Two children were infected with even lesser quantities of the virus (Table V-8). Schiff et al. (1983), concerned that most studies on minimal infective dose were done with vaccine strains of viruses, undertook similar studies with a wild strain of echovirus 12. Schiff's group infected 15 of 50 adults who ingested 10 PFU of echovirus 12 as titrated in $LLC\text{-}MK_2$ cells. The group also infected 9 of 20 adults fed 30 PFU of the virus (Table V-9).

TABLE V-5

Infections of Humans with the Attenuated SM Strain of Poliovirus 1*

Dose (PFU)	No. Infected/ No. Inoculated
0.2	0/2
2	2/3
20	4/4
200	4/4

*Source: Abstracted, revised and combined from Koprowski (1956) and Koprowski et al. (1956)

TABLE V-6

Infection of Infants with an Attenuated Strain of Poliovirus 3*

Dose ($TCID_{50}$)	No. Infected/ No. Inoculated
1	3/10
2.5	3/9
10	2/3
30-100	7/9
100-1000	4/4

*Source: Abstracted and composited from Plotkin and Katz (1967) and Katz and Plotkin (1967)

TABLE V-7

Infection of Infants with an Orally Administered Attenuated Strain of Poliovirus 1*

Dose ($TCID_{50}$)	No. Infected/ No. Inoculated
7	0/1
16	0/2
42	0/1
50	3/6
55	1/3
65	0/6
80	1/1
90	3/4
160	3/3
210	2/2
280	1/1

*Source: Abstracted and revised from Minor et al. (1981)

TABLE V-8

Infection of Children Intranasally with Attenuated Measles Vaccine*

Dose ($TCID_{50}$)	Children Infected/ Children Inoculated
0.1	0/21
0.2	1/7
0.6	1/8
1	8/35
2	14/29
6	18/26
10	18/21

*Source: Revised from Okuno (1962)

TABLE V-9

Infection of Healthy Adults with Echovirus 12*

Dose (PFU)	No. Excreting Viruses/ No. Inoculated	No. Seroconversions/ No. Inoculated	No. Infected/ No. Inoculated	Percent Infected
0	0/34	0/34	0/34	0
10	14/50	7/50	15/50	30
30	8/20	3/20	9/20	45
100	18/26	11/26	19/26	73
300	11/12	3/12	12/12	100

*Source: Abstracted and revised from Schiff et al. (1983) and Ward and Akin (1984)

Probit analysis of these data indicated a HID_{50} of 30 PFU and suggested that 1-2% of those ingesting 1 PFU would have been infected. Subsequent retitration of the virus in RD rhabdosarcoma cells by an improved technique indicated greater quantities of virus in the inoculum. With the new titration, the HID_{50} was ~900 PFU and the HID_1 ~30 PFU. Echovirus 12 is apparently an uncommon virus, however, and it is possible that it is a virus of relatively low infectivity. Morever, participants in the test were screened for antibodies by the hemagglutination-inhibition test, a relatively insensitive test. Thus, it is possible that many of the adults participating in the test had substantial quantities of antibody to the virus.

In conclusion, there are many strains of viruses that have not produced infections when administered in small numbers, and there are many strains of viruses that have produced infections when administered in small numbers. Our concern is about the latter strains of viruses.

VI. MECHANISMS OF DISEASE

Factors that Affect Disease Occurrence

Virulence. Many genetically-controlled factors affect the virulence of viruses, which is largely attributed to factors that promote the multiplication of viruses and bring about the destruction of the cells they infect. Virulence is greater for viruses that can multiply at fever temperatures. Fever is a frequent host response to infection, and those viruses that cannot multiply at fever temperatures cannot overcome their hosts in acute illnesses. Virulent viruses do not induce a great amount of interferon production, which is a major host defense mechanism in acute infections. Virulent viruses may also damage cell lysosomes and allow the escape of enzymes that may damage the cell. Virulent viruses may also block the synthesis of macromolecules and may alter the cell membranes of infected cells damaging them or bringing about their eventual phagocytosis. Viruses that have become attenuated produce little or no clinical disease.

The Susceptibility of Host Cells.

Cell Receptors -- When a virus infects a cell, it begins by adsorbing to a receptor moiety on the cell. If the cell has no receptor for the virus, the virus does not infect that cell; thus, many viruses do not infect cells in which they might be able to multiply. Polioviruses, for example, cannot infect the chick embryo because receptors for polioviruses are not present on chick embryo cells. Yet, if nucleic acid is carefully extracted from polioviruses and inoculated into chick embryos, the viral nucleic acid may gain entrance into cells of the chick embryo, multiply, and produce fully developed and intact polioviruses. These newly produced viruses cannot infect other cells in the chick embryo as long as the nucleic acid is contained within a viral capsid and require a specific receptor on a cell in order to infect it (Lennette and Schmidt, 1979).

Sometimes viral receptors exist on cells but require some form of activation before a given virus can adsorb to it and begin the infective process.

Maturation -- Sharp changes in susceptibility to viral infections may accompany the maturation of the host. Newborn mice, for example, are highly susceptible to coxsackievirus infections, but adult mice are considerably less susceptible or are not susceptible at all. The reduced or lost virulence is largely attributable to a loss of cell receptors with maturation. Poliomyelitis in adult humans is usually a much more severe disease than it is in young children. The same is true for chicken pox (Lennette and Schmidt, 1979).

Genetic Factors -- Certain diseases affect some strains of animals and not others. In other situations, relative severity of a disease may differ in different genetic populations.

States of Disease

Diseases produced by viruses may be apparent (clinical) or inapparent (subclinical). Apparent infections may be localized or disseminated.

Apparent Infections. Some viruses, such as measles, chicken pox and rubella, usually produce apparent infections.

Localized Infections -- Some viruses cause localized lesions. Warts, for example, may produce only local lesions. Even diseases such as gastroenteritis and influenza are essentially localized, affecting a single organ primarily. Sometimes viruses spread from the main target organ to distant organs, but such spread may not affect the character of the illnesses.

Disseminated Infections -- Disseminated infections follow a series of sequential steps. Thus, some viruses, after gaining entrance into the body, may multiply locally, spread to regional lymph nodes and multiply further, and then enter the lymphatics and the bloodstream producing a primary viremia. These viruses may then spread to other organs throughout the body. The viruses multiply in target organs and spill into the bloodstream producing a secondary viremia. The secondary viremia results in the transport of viruses to the main target organs. Polioviruses can produce disseminated infections of this kind (Lennette and Schmidt, 1979). The pathogenesis of poliomyelitis is described in Chapter IV.

Inapparent Infections. Inapparent infections are the most common manifestation produced by many viruses. Most enterovirus, adenovirus and reovirus infections are inapparent. For example, in two studies, symptoms were detected in only 8-28% of infants that were excreting rotaviruses (Totterdell et al., 1976; Murphy et al., 1977).

Inapparent infections produce an antibody response and consequently long-term immunity. However, individuals with inapparent infections are usually virus transmitters, and viruses transmitted by inapparently-infected carriers can be just as virulent as those transmitted by infected individuals who are acutely ill (Lennette and Schmidt, 1979).

Inapparent infections may be produced by virus strains that are attenuated. Sometimes most or all strains of a virus are attenuated or relatively attenuated and produce little clinical illness. Sometimes few strains of a virus are attenuated in nature, and consequently almost all infections, as with measles, result in clinical disease. Inapparent infections may also be produced in individuals who are immune from clinical illness as the result of earlier infections with the same virus. Immunity from clinical illness conferred by an earlier infection does not imply immunity from localized inapparent infections. Inapparent infections may also occur whenever a relatively virulent virus cannot reach a target organ in a susceptible host. An infection produced by small numbers of even highly virulent viruses in an initially nonimmune individual may be inapparent, because the host may rally sufficient defenses before the numbers of viruses reach levels sufficient to seriously damage target organs (Lennette and Schmidt, 1979).

Host Defense Systems

Antibodies. IgG antibodies circulating in the blood and in the extracellular fluids are the host's major defense against recurrence of a given disease, the antibodies having been produced following the initial infection with the virus. In addition, the GI tract and the respiratory tract are protected locally by IgA antibodies also produced in response to the initial infection with the virus. Usually, antibodies appear not to be an important factor in recovery from initial viral infections. Antibodies do not begin to appear in the blood for many days after infection begins and do not occur in high titers for several weeks after infection. Their production peaks usually after the infection has begun to subside. However, in two immunodeficient chronically symptomatic patients rotavirus excretion persisted for 6 weeks (Saulsbury et al., 1980). Factors other than antibodies usually bring about recovery from the initial viral infection. Nonetheless, it is possible to intercede artificially with antibodies and to bring about the abortion of some viral infections with long incubation periods. Both hepatitis A and rabies can be aborted by introducing antibodies into the host passively in the form of gamma globulin early in the incubation period. Antibodies persist at low levels long after viral infections subside, especially if viremia is produced (Lennette and Schmidt, 1979).

Cell-Mediated Immunity.

Cytotoxic T Lymphocytes (T Killer Cells) -- T killer cells recognize viral antigens on infected (target) cells, react with those cells, and destroy them. Enveloped viruses are more likely to elicit this response than nonenveloped viruses, because enveloped viruses impart antigens to the plasma membranes of the cells they infect. Group B coxsackieviruses, which are nonenveloped, and perhaps other nonenveloped viruses as well also bring about this response in cells they infect (Lennette and Schmidt, 1979).

Natural Killer (NK) Cell Cytotoxicity -- Natural killer cells are found in the peripheral blood of humans that are cytotoxic without the need for sensitizing antibodies. NK cells appear to be interferon-induced.

Nonspecific Factors in Immunity.

Interferons -- Interferons are small proteins produced by infected cells in response to viral infections. Interferons nonspecifically interfere with the infection of other cells by viruses by preventing the synthesis of viral proteins. Interferons are probably the single most important factor in bringing about recovery from an initial virus infection.

Macrophages -- Macrophages are scavenger cells that rapidly ingest viruses and eliminate them. Macrophages constitute an important nonhumoral defense factor and are important in bringing about the recovery of a host after initial infection with a virus.

VII. RISK ASSESSMENT

Introduction

The entire population is at risk of succumbing to waterborne viral infections in the United States. There are no clearly identified particularly vulnerable populations except for the immunocompromised, and there is no special geographic vulnerability. The number of reported enteric virus infections that are waterborne in the United States appears to be only a small percentage of the total number of enteric virus infections that occur. The large fraction of infections that are inapparent, and the extensive variety of symptoms produced by many enteric viruses when diseases do occur, may make detection of many waterborne outbreaks of enteric virus infections and diseases especially difficult. Yet, in recent years, as virus concentration and detection methodologies have become more sophisticated, viruses have been recovered more frequently at water intakes, and even in well-treated chlorinated drinking water supplies (sometimes in the absence of indicator organisms). Moreover, the occurrences in recent years of several large waterborne outbreaks of GI disease caused by the newly discovered Norwalk and Norwalk-like viruses now suggest that many earlier waterborne outbreaks of GI disease of unknown etiology were probably due to these viruses (Craun, 1979, 1981; Kaplan et al., 1982). Melnick and Gerba (1982) noted that if all gastroenteritis of unknown etiology were viral, 63% of all waterborne illnesses reported in the United States between 1971 and 1977 would have been related to the presence of viruses in drinking water.

Transmission of Viruses by Drinking Water

Reasons for Continuing Concern. Although epidemiological support for widespread transmission of viruses by the water route is still limited, concern about water transmission remains unabated. Akin (1977) and the AWWA Committee on Viruses in Drinking Water (1979) summarized reasons for that concern as follows:

"1. Human enteric viruses can survive for extended periods in the aquatic environment and these viruses have been readily isolated from wastewater and polluted surface water used as source water for treatment plants.

"2. Laboratory data have shown most enteric viruses to be more resistant than indicator bacteria to inactivation by water disinfectants.

"3. Infectivity tests have shown that human infections could be caused by one cell culture dose of poliovirus under experimental conditions.

"4. A consistently high endemic level of hepatitis A occurs in the United States and investigations have shown contaminated water to be a source of hepatitis A outbreaks.

"5. Sporadic outbreaks of nonbacterial gastroenteritis suspected of being waterborne occur rather frequently, and an endemic level of gastroenteritis occurs in the United States; waterborne viruses possibly may be responsible.

"6. Increased water demand is making direct recycling of wastewater almost a reality for some water systems, and conventional water treatment is not designed to produce potable water from wastewater."

Epidemiological Evidence. From 1946 to 1980, viruses were clearly responsible for almost 12% of all waterborne outbreaks of disease in the United States (Lippy and Waltrip, 1984) and probably for a large proportion of the >52% that were of unknown etiology. Most of the viral outbreaks from 1946 to 1982 were infectious hepatitis (73). Seventeen outbreaks (including several on cruise ships) were Norwalk virus gastroenteritis. At least eight waterborne outbreaks of rotavirus disease have occurred worldwide since 1977, with one of them in the United States (Gerba et al., 1984).

Tables VII-1 and VII-2 list all of the waterborne outbreaks of hepatitis A and Norwalk and Norwalk-like gastrointeritis that occurred in the United States from 1973 to 1982 (CDC, 1974, 1975, 1976, 1977, 1979, 1980, 1981, 1982a,b, 1983). During that period, most outbreaks of hepatitis A and Norwalk and Norwalk-like gastroenteritis involved noncommunity and individual water supplies. Only ~40% of the outbreaks involved community supplies (Table VII-3). A single waterborne outbreak of rotavirus-induced GI disease was associated with a community surface water (stream) supply (CDC, 1982b). Most of the Norwalk virus-induced outbreaks reported have been common source and most of those outbreaks in which the source was determined were waterborne (Kaplan et al., 1982). Thus, the major mode of transmission for Norwalk viruses may be drinking water.

The first known probable waterborne outbreak of Norwalk virus gastroenteritis occurred in 1969. Undoubtedly, many such outbreaks occurred in earlier years, but were not reported because the Norwalk and Norwalk-like viruses had not yet been discovered. The 73 epidemics of infectious hepatitis produced only 2415 cases of illness; the 17 epidemics of Norwalk virus gastroenteritis produced 3692 cases of illness. Goldfield (1976) lists 30 epidemics that produced more than 20,000 cases of nonbacterial gastroenteritis from 1965-1974. In addition, in just one waterborne outbreak in Georgetown, Texas reported recently, ~8000 cases of nonbacterial gastroenteritis occurred in advance of a hepatitis A outbreak (Hejkal et al., 1982).

TABLE VII-1

Waterborne Outbreaks of Hepatitis A in the United States from 1973-1982[a],

Date	State	No. of Cases	Water System	Water Source	Location of Outbreak	Cause of Outbreak
June 1982	GA	10	Individual	Well	Day-care center	Untreated groundwater
July 1982	GA	35	Community	Well	Trailer park	Untreated groundwater
November 1982	KY	58	Community	Spring	Community	Untreated groundwater
October 1980	PA	48	Individual	Well	Rural area	Deficiencies in treatment
June 1980	TX	36	Community	Well	City	Deficiencies in treatment
July 1980	WI	3	Individual	Well	Plant	Untreated groundwater
July 1977	SC	47	Noncommunity	Unavailable	Factory	Deficiencies in distribution system
February 1975[b]	MA	17	Individual	Unavailable	Unavailable	Untreated surface water
February, March 1973	AL	50	Community	Unavailable	Unavailable	Deficiencies in treatment
July, August 1973	OH	35	Noncommunity	Unavailable	Unavailable	Untreated groundwater

[a] Source: Abstracted and revised from CDC (1974, 1975, 1976, 1977, 1979, 1980, 1981a, 1982a,b, 1983)

[b] Reported as hepatitis

TABLE VII-2

Waterborne Outbreaks of Norwalk and Norwalk-Like Virus Diarrhea in the United States from 1978-1982*

Date	State	No. of Cases	Water System	Water Source	Location of Outbreak	Cause of Outbreak
May 1982	AL	55	Community	Wells	Community	Deficiencies in distribution system
April 1982	CA	25	Community	Storage tank	Reservation	Deficiencies in treatment
April 1982	CA	170	Community	Broken main	USMC depot	Deficiencies in distribution system
January 1982	GA	500	Community	Spring	Community	Untreated surface water
January 1980	CA	190	Community	Lake	Resort Community	Deficiencies in treatment
August 1980	GA	1500	Community	Springs	City	Possible deficiencies in distribution system
July 1980	MD	127	Noncommunity	Well	Camp	Deficiencies in treatment system
August 1980	NY	42	Noncommunity	Well	Campground	Untreated groundwater
July 1980	PA	55	Noncommunity	Well	Restaurant	Deficiencies in treatment
October 1979	NC	145	Noncommunity	Ground	Camp	Untreated groundwater
July 1979	PA	151	Noncommunity	Ground	Camp	Untreated groundwater
May 1978	PA	350	Noncommunity	Well	Camp	Deficiencies in treatment
July 1978	PA	120	Noncommunity	Well	Camp	Deficiencies in treatment
May 1978	WA	467	Noncommunity	Well	School	Deficiencies in treatment system

*Source: Abstracted and revised from CDC, 1974, 1975, 1976, 1977, 1979, 1980, 1981, 1982a,b, 1983)

TABLE VII-3

Water Systems Involved in Outbreaks of Viral Disease in the United States, 1973-1982[a]

Year	Virus	No. of Outbreaks (No. of cases)			Total No. Outbreaks	Total No. of Cases
		Community Water systems	Non Community Water System	Individual Water System		
1982	Hepatitis A	2 (93)	0	1 (10)	3	103
	Norwalk, Norwalk-like	4 (750)	0	0	4	750
1981	Rotavirus	1 (1761)	0	0	1	1761
1980	Hepatitis A	1 (36)	0	2 (51)	3	87
	Norwalk, Norwalk-like	2 (1690)	3 (224)	0	5	1914
1979	Norwalk, Norwalk-like	0	2 (296)	0	2	296
1978	Norwalk, Norwalk-like	0	3 (937)	0	3	937
1977	Hepatitis A	0	1 (47)	0	1	47
1975	Hepatitis A	0	0	1 (17)	1	17
1973	Hepatitis A	1 (50)	1 (35)	0	2	85
SUBTOTALS	Hepatitis A	4 (179)	2 (82)	4 (78)	10	339
	Norwalk and Norwalk-like	6 (2440)	8 (1457)	0	14	3897
	Rotavirus	1 (1761)	0	0	1	1761
TOTALS		11 (4380)	10 (1539)	4 (78)	25	5997

Source: Derived from Tables VII-1 and VII-2 and from sources listed therein

Kaplan et al. (1982) analyzed 38 confirmed Norwalk virus gastroenteritis outbreaks (reported to the CDC or to the National Institutes of Health) that occurred between 1967 and 1980. All but seven of the outbreaks occurred in the United States. Three of the outbreaks occurred in other countries; four occurred on cruise ships. A common source was indicated in 31 of the 38 outbreaks, and a vehicle of transmission was identified in 17 of these. Water was the vehicle in 13 of the outbreaks, food in four. Primary person-to-person transmission was responsible for only seven of the outbreaks. These data seem to suggest that most Norwalk virus gastroenteritis is water-transmitted. Since only a few thousand cases of illness are attributable to these reported outbreaks, and since more than half of the adults in the United States have antibodies to Norwalk virus (Kapikian et al., 1978, 1982), it would appear that numerous undetected waterborne outbreaks of Norwalk-virus gastroenteritis must occur. It is likely that most waterborne disease outbreaks are not detected and not reported. Martin Goldfield's description (Goldfield, 1976) of his first awareness of a massive waterborne epidemic during his tenure as Assistant Commissioner of the New Jersey State Department of Health is particularly instructive:

> "... in June, 1966, I received a phone call from a worried school nurse at one of the community's elementary schools who reported that two-thirds of the pupils and two of three teachers of three fifth grade classes were absent that day. Her major concern was that she had been instructed by superiors not to report the event, and she begged for confidentiality. It was quickly and easily ascertainable that we were dealing with a common source waterborne outbreak of gastroenteritis associated with a park located in neighboring Pennsylvania, that it was caused by a transmissable

> agent, and that the outbreak had undoubtedly been huge, since interviews of all groups registered with park officials revealed an average attack rate of more than 60%. It could be estimated that about 13,000 people became ill after drinking water at the park during a four-day interval. Yet, had it not been for the phone call from the school nurse, this outbreak, and its source, would probably have gone unrecognized. The question we must ask ourselves, therefore, is how many large, dramatic common source outbreaks do we fail to recognize? Regrettably the answer must be 'many'."

This anecdote not only describes a massive (13,000 cases) disease outbreak that was waterborne and quite possibly viral, but one that some authorities tried to hide (almost successfully despite its enormous size), and which would have escaped detection if the school nurse had not drawn attention to it. Every year, most communities usually experience several epidemics -- "something is going around." Yet, although thousands of cases must occur to bring about an awareness that "something is going around," the source of the outbreak is almost never questioned.

> "The general public -- and even large segments of the medical community -- tend to have a grossly distorted impression with regard to surveillance of disease in the United States Over the years, they have been led to believe that some system exists for the recognition of outbreaks which provides reasonable assurance that significant clusters of illness will be recognized, studied and handled appropriately by public health authorities. To them, the absence of reported outbreaks provides reassuring evidence of our safe and healthful environment. Unfortunately, this is pure myth. In my own experience, even huge waves of febrile respiratory disease, gastrointestinal illness, measles, or german measles can go completely undetected unless arduous efforts are made to develop surveillance programs ..." (Goldfield, 1976).

The widely held view is that currently available epidemiological techniques are not sufficiently sensitive to detect waterborne transmissions (Report to Congress, 1978; AWWA Committee on Viruses in Drinking Water, 1979).

Rotaviruses have been implicated in waterborne outbreaks only recently. These viruses are the etiological agent of most acute gastroenteritis in infants 6-24 months of age (Kapikian et al., 1976; Brandt et al., 1983). At least one such outbreak occurred in the United States (Gerba et al., 1984). About half of the most severe disease seen in hospitalized infants is caused by rotaviruses (Kapikian et al., 1976; Brandt et al., 1983). These viruses are the leading cause of death in developing countries. In the United States, rotavirus infections are widespread. In a recent study in the Washington, DC area, 90% of the children tested had been infected with a rotavirus by the age of 3 (Brandt et al., 1983). Recovering rotaviruses from water requires techniques that have been developed only recently and are just now being applied in the field. Some recoveries of rotaviruses from drinking water have been made.

Viruses at Water Intakes and in Drinking Waters. Once viruses are discharged into receiving waters, their numbers begin to diminish slowly, the result of dilution and exposure to hostile chemicals and microflora in the water environment. Viruses do not multiply outside of susceptible living cells. Thus, by the time viruses discharged with sewage reach downstream water intakes, the numbers of surviving viruses are reduced to levels that, with the best techniques available, are barely detectable in 1000 ℓ quantities of water. However, the efficiency of the best detection techniques available is <20% with polioviruses (Report to Congress, 1978; Berg, 1978b). Moreover, the cell culture systems commonly used for detecting viruses are sensitive to <50% of the human types of viruses occuring in water (Berg, 1978b). The plaque technique, used for recovering and quantifying viruses, is relatively inefficient. Also it is likely also that viruses excreted by man into sewage have yet to be discovered. Thus, it is certain that the numbers of viruses detected at water intakes are considerably smaller than the numbers present. In terms of the daily water requirements of the community, however, even the number of viruses detected is not small (Berg et al., 1984).

With the technology available, viruses are readily and frequently detected along rivers and at water intakes. In studies along the Missouri and Mississippi Rivers, for example, viruses were recovered from 18 sites along the rivers; nine of those sites were at or near water intakes. At four sites, fecal coliform levels met recreational water standards. Three of those four sites were at or near water intakes (Berg and Metcalf, 1978).

Viruses have also been recovered from drinking waters. Twenty-three reports of such recoveries (see Table III-11) have been published (Melnick and Gerba, 1982; Berg, 1984). Many of these drinking waters had received complete treatment, and it was possible to verify the quality of treatment for some of these waters. It is important to underscore that viruses were recovered from fully treated drinking waters, and that some of these waters were known to have met bacteriological and turbidity standards. In a recent study on one such chlorinated drinking water, rotaviruses were recovered from 3 of 26 drinking water samples (100 ℓ) that were negative for total coliforms and contained turbidities of $\leq$1 NTU. The numbers of rotaviruses in the drinking water were 233, 125 and 78 infective units (foci) per 100 ℓ sample (Gerba, 1984).

Minimal Infective Dose. Only one virion is needed to infect one cell and to bring about multiplication of that virion (Beard, 1967), providing that the cell is susceptible and the virion is intact. Defective viruses may require a multiplicity of infections in order for multiplication of a virus to occur, and even a fully viable virus particle must reach a susceptible cell and find an intact receptor. There are many other circumstances that may interfere with the infection process or alter it. For example, in laboratory preparations (for reasons that are not understood), the number of noninfective virions may exceed the number of infective virions by several orders of magnitude, and noninfective virions may block receptors on cells and prevent infection. However none of these circumstances alter the basic fact that only one viral particle is needed to infect one susceptible cell.

The question that needs to be answered is: "Is the number of virus particles detected at a water intake or in a drinking water sufficient to produce an infection in an individual who consumes that number?" To answer this question, it is necessary to feed to individuals the number of viruses recovered from water. As an alternative, viruses have been inoculated (by feeding or otherwise) into individuals in numbers usually determined in the most susceptible cell cultures available. Studies should demonstrate that a small number of infective virus particles or clumps of particles (the numbers of which are determined in the sensitive cell cultures used to recover viruses from waters) can produce infection in a significant number of the individuals inoculated. The difficulties in obtaining such data are obvious. There is a risk in inoculating volunteers even with apparently innocuous viruses. Thus, only a few such studies exist. However, sufficient data are available to make the decisions on standards for viruses in drinking waters. Tables V-5 to V-8 show that viruses in numbers as small as 1 $TCID_{50}$ can infect a significant proportion of the individuals that consume that quantity. Two PFU of attenuated poliovirus 1(SM) in gelatin capsules infected 2 of 3 adult volunteers (see Table V-5); 3 of 10 infants fed 1 $TCID_{50}$ of attenuated poliovirus 3(Fox) by nasogastric tube were infected (see Table V-6); 3 of 6 two-month-old infants fed 50 $TCID_{50}$ of attenuated poliovirus 1 were infected (see Table V-7); 8 of 35 children were infected with 1 $TCID_{50}$ of measles virus administered by intranasal spray (see Table V-8). Even when larger numbers of viruses appear to be necessary to infect individuals that have been inoculated, extrapolation of the data often shows that one cell culture infective dose will still infect significant numbers of individuals.

The Need for Standards

The arguments for standards are based largely on the reported waterborne outbreaks, the frequent detection of viruses at water intakes (and in groundwaters), on the recoveries of viruses from chlorinated drinking waters, on the small numbers of viruses that often constitute the infective dose, and on the frequent recovery of viruses from waters that do not yield coliform organisms.* The arguments against standards are based largely on the lack of epidemiological evidence for transmission of viruses other than hepatitis A by the water route and the cost for the procedures necessary to monitor for viruses and to enforce standards (IAWPRC Study Group on Water

---

*The indicator problem will be dealt with in Chapter IX.

Virology, 1983). However, waterborne outbreaks of rotavirus and Norwalk and Norwalk-like virus disease are now known to occur (Gerba et al., 1984).

Magnitude of the Problem. The numbers of viruses detected in raw waters at water intakes and in other waters destined for human consumption have always been small, but the current state of technology is such that the numbers of viruses actually present exceeds the numbers of viruses detected (Report to Congress, 1978; Berg, 1978b). Thus, the magnitude of the problem is greater than available numbers might make it seem. Nonetheless, it is unlikely that the numbers of viruses present at water intakes or in groundwaters could be large enough to infect large numbers of individuals, unless there is a breakdown in treatment processes. Thus, the number of individuals infected by the water route is likely to be small by comparison with the numbers of persons infected by the personal contact route.

Mode of Transfer of Enteric Infections. If a comparison of numbers was the major reason for dispute, the water route would be of little significance. However, although 123 or more enteric viruses of man are known, only a small number of these viruses appear to occur in any one community at any one time. The occurrence in a community at any given time of only a small number of the existing enteric viruses is probably accounted for in large part by the immune status of the community's population (primarily of its child population), the generally reduced rate of transmission that occurs during the colder months for most viruses that are transmitted by the fecal-oral route, and during the warmer months for these viruses that are transmitted by the respiratory route. The immune status of a community's population to a given enteric virus usually depends on the time elapsed since that virus last circulated in the community, because the susceptibles are largely members of the young school age population and their younger siblings. Once most viruses (enteric or other) have circulated through such a population, these viruses are not likely to obtain a foothold in the community again until a population of new susceptibles has developed (new births and attainment of school age at which time the number of contacts is highest). It is possible that some viruses remain in a community endemically for long periods, maintained perhaps by passage through a relatively small number of susceptibles in the community. The virus causing a significant outbreak probably enters the community from without.

Personal Contact Transmission from Community to Community. It is possible in this age of great mobility for people who move from community to community to be transmitters, but this highly mobile population consists primarily of adults in commerce, a group of generally low level transmitters. The major transmitters, children of school age, are not usually highly mobile.

The Water Transmission Scenerio. To many proponents of waterborne transmission, a single plausible scenario for transmission envisions a single virus particle or clump of particles, entrapped in a minute bit of cellular debris, fecal matter, or other detritis, surviving water treatment processes within the small amount of suspended solids that passes through treatment, protected from disinfection by encapsulation within the solids, ingested by a susceptible individual (probably a child), and producing an asymptomatic infection in that individual. Once introduced into a susceptible community, the virus is readily transmitted by the personal contact route and produces a large scale outbreak of infection and disease (Berg, 1978; AWWA Committee on Viruses in Drinking Water, 1979). Although the number of virus particles ingested may be very small (perhaps only one), data are available that show that only one viral particle may be sufficient to produce an infection in a susceptible individual (Beard, 1967; Plotkin and Katz, 1967; Katz and Plotkin, 1967; Westwood and Sattar, 1976; Ward and Akin, 1984).

Other Considerations. Wellings et al. (1973) argued eloquently "...that man should not ingest a single virus particle unnecessarily..." but that "...the establishment of a virus standard for potable water per se would not assure virus free water. On the contrary," she continued, "it might lead to a false sense of security." Wellings based her argument on the lack of sensitivity and reliability of test procedures for viruses in potable and other waters, problems of enforcement, and on the availability of an alternate approach.

The IAWPRC Study Group on Water Virology (1983), while acknowledging the evidence for waterborne transmission of infectious hepatitis and gastroenteritis, noted that the low-level transmission hypothesis is still unproven, and while there is evidence that the minimal infective dose for some viruses consists of a small number of viruses, that there is little evidence that it is one viral particle. Moreover, monitoring techniques for viruses in water are inefficient and expensive. However rather than totally discounting the importance of water transmission, the group conservatively advocated research to determine the minimal infective dose for viruses, to improve systems that are economic for monitoring viruses, and to obtain more information on the epidemiology of waterborne viral diseases.

Some of the same arguments had been made earlier by Gamble (1979) who questioned also whether consumption of 1 PFU of virus was hazardous, and argued that without information relating the extent of viral contamination of drinking water to disease, there was no logical basis for a standard.

Wellings et al. (1973) noted that virus recovery techniques lacked precision and accuracy [they still do today although efforts are being made to improve them (Berg, et al., 1984; Melnick et al., 1984)], that many viruses were not recovered by the cell culture systems used to detect viruses (which resulted in significant underestimation of the number of viruses present), and that results became available so slowly, it was likely that waters would be consumed before it was known that they were contaminated. All of this is still true today. Unfortunately, total coliform tests may not be much more accurate than available virus recovery tests. Not only are total coliform membrane filter procedures susceptible to many false positives and false negatives (Hendricks, 1978), but the time and temperature conditions allowed for transport of samples from field collection stations to the laboratory permit considerable die-off (and sometimes growth) of total coliform organisms and thereby considerable error in estimation of actual numbers present (McDaniels and Bordner, 1983).

The IAWPRC Group did not define an acceptable viral infection risk for water that was to be consumed by man. Many of the Group argued that sterilization was impractical, and that "...risk should be evaluated on the basis of the contribution of water to the overall incidence of viral disease, and that the expenditure on water treatment and quality control should be weighed against the possible diversion of funds into revenue that may more successfully aid public health in general" (IAWPRC Study Group on Water Virology, 1983).

Recommended Standards for Viruses in Waters (non-U.S. EPA)

Drinking Waters (non-U.S. EPA). In 1971, the position was presented that there should be no viruses in any waters consumed by man (Berg, 1971). In that same year, Melnick (1971) suggested a virus standard for potable waters of no more than 1 PFU per 100 gallons of water. During the next year, the World Health Organization (WHO) recommended a limit for viruses in drinking water of zero viruses in 10 ℓ of water (WHO, 1972).

Later, Shuval (1976) proposed a standard of zero viruses in 100 gallons of potable water. Shuval suggested that such a standard might not be good enough, but that it would upgrade significantly the quality of water throughout the world. By 1976, Melnick felt that monitoring technology had improved sufficiently to permit a virus standard for potable waters of 1 PFU per 1000 gallons of water (Melnick, 1976). Melnick argued that we should not wait for precise measurements on the numbers of viruses in water that might produce disease, and that the community is entitled to drink water as free from viruses as current technology can provide. Shuval (1976) argued that coliform standards were set long before precise information was available on the numbers of coliforms that correlate with disease. In fact, good correlations still do not exist. Lennette (1976) suggested that a temporary standard for viruses in water should be recommended, but argued that an atmosphere of crisis should be avoided.

In 1979, a WHO Scientific Group recommended that drinking water should be free from human enteric viruses and that to achieve this goal 100-1000 ℓ samples of water should be tested by the most sensitive method available. The group asserted that "In all cases of intentional direct wastewater reuse for domestic consumption, this procedure should be considered essential and should be applied at least in large urban areas in which potable supplies are derived from virus-polluted sources, such as surface water containing a significant proportion of wastewater either untreated or insufficiently treated to inactivate viruses ..." (WHO Scientific Group, 1979).

In its drinking water guideline, the WHO echoed its earlier assertions that "... drinking water should be free from any viruses infectious for man..." but argued that "Adequacy of treatment cannot be assessed in an absolute sense because neither monitoring technique nor epidemiological evaluation is sufficiently sensitive to ensure the absence of viruses or the freedom from endemic occurrences of waterborne infections ..." (WHO, n.d.). Shortly before this WHO report was rendered, an expert group of 19 environmental virologists in a report to the U.S. EPA echoed The WHO Scientific Group (1979) decision and recommended to U.S. EPA that a limit of <1 virus/1000 ℓ should be set for drinking water (Conference on Monitoring for Viruses in the Environment, 1981). Application of the standard would follow the WHO Scientific Group's recommendation (WHO Scientific Group, 1979).

Renovated Waters (non-U.S. EPA). In 1973, the WHO recommended that each batch of renovated water should be monitored for viruses before the water is released to the public (WHO, 1973). Shuval recommended that in such waters no enteroviruses should be detected in 100 gallon samples and when it becomes possible to monitor larger samples, that the standard should be upgraded accordingly (Shuval, 1976). The WHO Scientific Group (1979) recommended that there should be no viruses in 100-1000 ℓ samples of such waters (see above). The Conference on Monitoring for Viruses in the Environment (1981) recommended standards for renovated waters that were essentially the same as those of the Scientific Group's recommendation (see above).

Limits have been set for enteric viruses in recreational waters and agricultural irrigation waters by the State of Arizona (1981) (Table VII-4).

The Bacteriological Standard for Drinking Waters

The total coliform test is the basis for the bacteriological standard for the microbiological safety of drinking water. Consideration for a viral standard implies the inadequacy in the coliform standard's ability to safeguard against a viral presence, since viruses have been recovered from drinking waters that were negative for total coliforms in standard tests.

Total coliforms have no sanitary significance. Most of the bacteria that constitute the total coliform group are ubiquitous in nature and unassociated with human wastes. The fecal coliforms, which are associated with feces, constitute only ~10% of the total coliform numbers in sewage. The use of a fecal coliform standard for drinking water would have clear sanitary significance. However, a total coliform standard is used because of the large margin of safety (~10-fold) its use tenders as compared with the fecal coliforms alone since both groups are about equally affected by most treatment procedures and about equally sensitive to disinfection.

TABLE VII-4

Limits for Enteric Viruses and Fecal Coliforms in Effluent Dominated Waters in the State of Arizona[a]

Organism	Recreational Water		Agricultural Irrigation
	Full Body Immersion	Partial Body Immersion	
Enteric Viruses[b]	1 PFU/40 ℓ	125 PFU/40 ℓ	1 PFU/40 ℓ
Fecal Coliforms[c]	200 units/100 mℓ	1000 units/100 mℓ	Variable with circumstance

[a] Source: State of Arizona (1981)

[b] Maximum allowable limit, mean (5 sample minimum)

[c] Maximum allowable limit, geometric mean (5 sample minimum)

The absence of coliforms is supposed to reflect the absence of bacterial pathogens. Since bacterial pathogens are almost always present in sewage in smaller numbers than fecal coliforms and since both are removed to about the same degree by treatment procedures and inactivated one about as quickly as the other by chlorine, a fecal coliform standard equivalent to the present total coliform standard for drinking water would probably be adequate to ensure the absence of bacterial pathogens from drinking water. The use of the total coliform standard adds about a 10-fold margin of safety. Thus, we have gone to great lengths to insure the absence of even one bacterial pathogen from drinking waters although we know that with most of these pathogens, infection and disease of a single individual requires the ingestion of at least hundreds and usually thousands of these organisms (Akin, 1983). However, we seem unsure that we must keep drinking water free from viruses, although the ingestion a of small number of viruses can produce infection.

Appraisal of Risk

An appraisal of the risk imposed by the presence of small numbers of viruses in drinking waters is difficult to make and requires certain suppositions. Such an appraisal has been made by Gerba (1984) and is presented below.

> "Long and Bell (1972) calculated that if a water supply contains one plaque-forming unit of virus per 190 liters of drinking water in a community using 190 million liters per day (0.2% of which is ingested as drinking water), assuming a 30% infection rate, 600 individuals would be affected daily by a variety of clinical and subclinical infections. If two liters per day is assumed to be average per capita consumption of drinking water, this would represent a community of 190,000 people. Thus, using the assumptions of Long and Bell, we would expect 219,000 infections to occur in the community over a period of one year, or more than one infection per person. Dingle et al. (1953) found

> that gastroenteritis accounts for 15% of all illnesses observed in families and that the average number of episodes per individual was 1.6 per year. Over the three year study period, the average number of episodes ranged from 1.5 to 1.7.
>
> Shown in Table VII-5 are the numbers of illnesses that would be expected in a community of 190,000 over a one year period of time, assuming various concentrations of enteric viruses in the water supply and different rates of infection. If it is assumed that every infection results in an overt case of gastroenteritis, an individual consuming 1 PFU of virus per 1000 liters of water would experience 0.0073 cases of disease per year. At an infection rate of 10%, this would be 0.073 cases, and at an infection rate of 30% it would be 0.22 cases per year. Based on these assumptions, allowing less than 1 PFU of virus per 1000 liters of drinking water produce no easily observable impact on the incidence of gastroenteritis within a community."

Recent studies with adult volunteers indicate that between 1 and 10% of the individuals consuming 1 PFU of a strain of echovirus 12 became infected (Akin, 1983). Other studies with children showed that 30% of the individuals ingesting 1 PFU of a strain of poliovirus 1 became infected (Plotkin and Katz, 1967; Katz and Plotkin, 1967). In the latter studies, the virus was administered by a gavage tube, and this could be considered a worst case. These studies did not consider that infection rate probably varies with the type of virus.

Gerba (1985) also projected infection rates in a hypothetical community whose water supply contained viruses (Figure VII-1). Gerba's projections were based on data in the literature (Katz and Price, 1967; Minor et al., 1981; Schiff et al., 1983). The data were fit by the method of Haas (1983).

Summary

Hepatitis A virus, Norwalk and Norwalk-like viruses, and rotaviruses have been implicated in many waterborne outbreaks of disease. Hepatitis A

TABLE VII-5

Virus Concentrations in Water and Expected Illness[a]

Assuming a Standard of 1 PFU per Volume Shown	Number of Illnesses per Year Assuming an Infection Rate of			Incidence of Infection per 100 Persons Assuming an Infection Rate of			Number of Illnesses[b] per Person per Year Assuming an Infection Rate of		
	1%	10%	30%	1%	10%	30%	1%	10%	30%
10 ℓ	138,667	1.4 x 10^6	4.16 x 10^6	73	100	100	0.73	7.3	22
100 ℓ	13,867	140,000	416,000	7.3	73	100	0.073	0.73	2.2
190 ℓ	7,300	73,000	219,000	3.8	38	100	0.036	0.38	1.15
380 ℓ	3,650	36,500	109,500	1.9	19	57	0.019	0.19	0.57
1000 ℓ	1,387	13,867	41,600	0.73	7.3	22	0.0073	0.073	0.22
1900 ℓ	730	7,300	21,900	0.38	3.8	11.5	0.0036	0.038	0.11

[a] Source: Gerba (1984)

[b] In a community of 190,000 individuals

NOTE: This table assumes that 2 ℓ of water are ingested per person per day.

virus is an etiological agent of infectious hepatitis, while Norwalk virus, Norwalk-like viruses, and rotaviruses are responsible for GI illnesses. Moreover, drinking water appears to be an important route for the transmission of the Norwalk virus.

In recent years, as methods for detecting viruses in water have improved, viruses have been frequently recovered at water intakes and from drinking waters, sometimes from well-treated chlorinated supplies, and sometimes in the absence of indicator organisms. At least 23 reports of recoveries of viruses from drinking waters have been published. Hepatitis A viruses and rotaviruses have both been recovered from drinking waters. With many viruses, the number necessary to produce infection in an individual who ingests them is small and may be as small as one.

In all, from 1946-1982, 73 outbreaks of infectious hepatitis have been reported in the United States. Seventeen outbreaks of Norwalk virus gastroenteritis and one outbreak of rotavirus gastroenteritis have been reported in the United States in the few years since these viruses were discovered. Most of the waterborne outbreaks in the United States from 1973-1982 were groundwater-related. Eleven (including the rotavirus outbreak) were derived from community supplies and 10 from noncommunity supplies. Only four were derived from individual supplies. However, the sources of most outbreaks of disease were not determined.

Arguments in behalf of standards for viruses in drinking waters are based on the occurrences of the reported waterborne outbreaks, the frequent detection of viruses at water intakes and in groundwaters, the growing

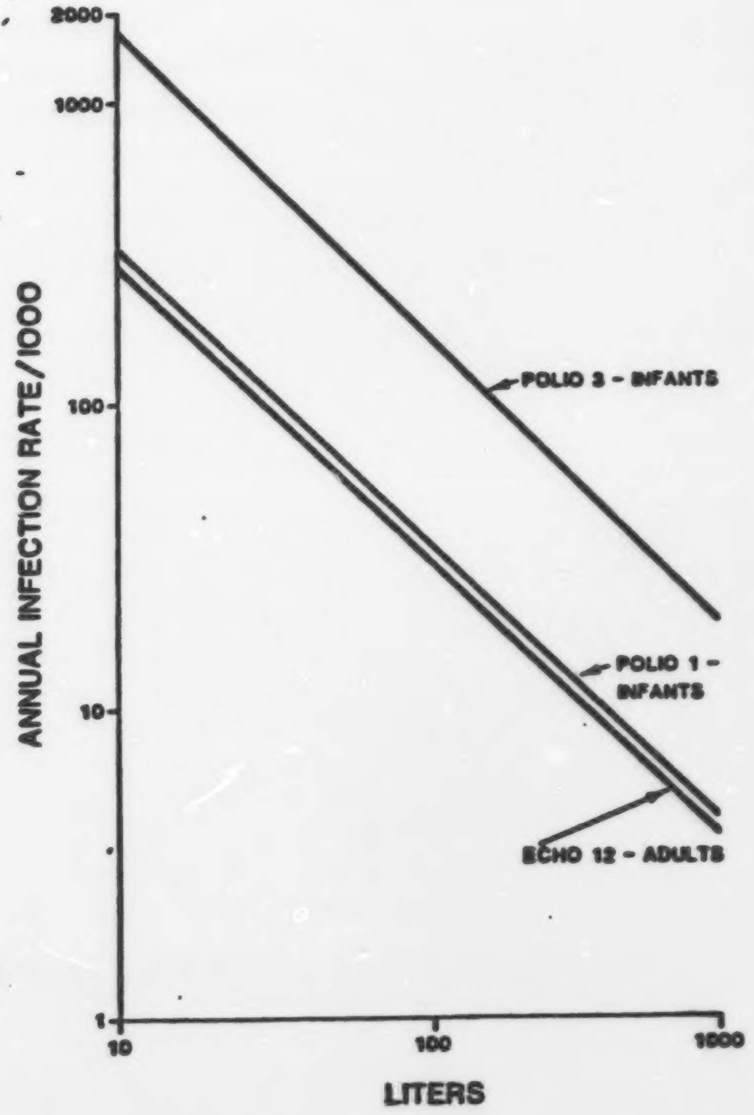

FIGURE VII-1

Expected Number of Infections Resulting from the Presence of One Infective Virus Particle in the Volume of Water Indicated

Source: Gerba, 1985.

numbers of virus recoveries from chlorinated drinking waters (often in the absence of coliform organisms), and on the small numbers of viruses that often constitute the infective dose. The arguments against standards are based largely on the absence of data indicating waterborne viral disease from properly treated water supplies, a lack of epidemiological evidence for the transmission of viruses other than hepatitis A virus by the water route (however, many waterborne outbreaks of Norwalk virus, Norwalk-like virus, and rotavirus gastroenteritis have been reported in recent years), and on the cost necessary to monitor for viruses and to enforce standards.

Several expert groups in the United States and elsewhere have urged that there should be no viruses in drinking water and have recommended standards to meet that goal.

VIII. METHODOLOGY

Introduction

In the past two decades, as interest developed in viruses that appeared to be present in environmental waters, methods for recovering these viruses were developed. A wide assortment of approaches led to the early combination of a large number of methods. Included among them were filter adsorption-elution and adsorption to inorganic precipitates, polyelectrolytes, minerals and clays, glass beads, and protamine sulfate (Table VIII-1). Methods that depended upon ultrafiltration, hydroextraction and reverse osmosis were also developed.

As the years passed, many of the methods seemed abandoned as other apparently better methods were developed. Eventually, most of the abandoned methods were resurrected and often found use as secondary concentration methods.

Today, the best methods for drinking waters appear to be filter-adsorption elution techniques that concentrate viruses from volumes of 4000 ℓ or more of tap waters into volumes of ~2 ℓ followed by a reconcentration procedure that reduces the final volume of concentrate to a few milliliters.

Methods for Concentrating and Recovering Viruses from Drinking Water

Filter adsorption-elution methods have gone through many phases of development (Wallis and Melnick, 1967; Berg et al., 1968a, 1971; Hill et al., 1972; Wallis et al., 1972; Jakubowski et al., 1974, 1975; Farrah et al., 1976; APHA, 1980) since Metcalf (1961) reported the first membrane

TABLE VIII-1

Methods for Concentrating Viruses from Water*

Method	Volume of Water Processed	Applications	Remarks
Filter adsorption-elution Negatively charged filters	large	all but very turbid waters	Best system for concentrating viruses from large volumes of tap water, sewage, seawater and other natural waters. Cationic salt concentration and pH must be adjusted before processing.
Positively charged filters	large	tap water	These filters have only been tested with tap waters, but they may be useful for other types of water as well. No preconditioning of waters may be necessary.
Adsorption to metal salt precipitate, aluminum hydroxide, ferric hydroxide	small	tap water, sewage	Have been useful as reconcentration methods.
Polyelectrolytes - PE60	large	tap water, lake water, sewage	Because of its unstable nature and lot-to-lot variations in efficiency for concentrating viruses, the method has not been used in recent years.
Bentonite	small	tap water, sewage	Can be used in a sandwhich between filter paper supports to process up to 100 ℓ volumes.
Iron oxide	small	tap water, sewage	
Talcum powder	large	tap water, sewage	
Gauze pad	large	--	First method developed for detecting viruses in water, but not quantitative.
Glass powder	large	tap water	Columns containing glass powder have been made that are capable of processing 400 ℓ volumes.
Organic flocculation	small	reconcentration	Widely used method for reconcentrating viruses from primary filter eluates.
Protamine sulfate	small	sewage	Efficient method for concentrating reoviruses and adenoviruses from small volumes of sewage.
Polymer two-phase	small	sewage	Processing is slow; method has been used to reconcentrate viruses from primary eluates.
Hydroextraction	small	sewage	Often used as a method for reconcentrating viruses from primary eluates.
Ultrafiltration			
Soluble filters	small	clean waters	Clog rapidly even with low turbidity.
Flat membranes	small	clean waters	Clog rapidly even with low turbidity.
Hollow fiber or capillary	large	tap water, lake water	Up to 100 ℓ have been processed, but water must often be prefiltered.
Reverse osmosis	large	clean waters	Also concentrates cytotoxic compounds that adversely affect assay methods.

*Source: Revised from Gerba (1983)

filter technique and Cliver (1967) developed a membrane filter adsorption technique. The methods derived from these efforts are capable of accommodating more than 4000 ℓ of drinking water and recovering many of the viruses contained in that water. Methods available today use either negatively or positively charged microporous filters. Negatively charged filter procedures have been standardized by Standard Methods (APHA, 1980) and by the U.S. EPA (Berg et al., 1984). The positively charged filters may have some advantages over the negatively charged filters, but sufficient experience with positively charged filters is not yet available.

Drinking water samples may be processed in the field or in the laboratory. Since volumes of 4000 ℓ or more may need to be processed, it is usually most practical to process in the field with a portable virus concentrator near the source (tap) to be sampled. If samples must be transported to the laboratory for processing, the samples must be dechlorinated immediately upon collection and transported under refrigeration.

Negatively Charged Virus-Adsorbing Filters. Standardized negatively charged virus-adsorbing filter procedures for recovering viruses from tap waters have been published (APHA, 1980; Berg et al., 1984), and one procedure has undergone round-robin testing (Melnick et al., 1984). This term refers to tests done under identical conditions by several participating laboratories to determine the effectiveness, precision and accuracy of a method.

In these procedures, drinking water (chlorine neutralized) is acidified with hydrochloric acid to bring the pH to 3.5, and sufficient aluminum chloride is added to bring the concentration of that salt in the water to 0.0005 M. The acidified, salted water is passed through a mixing chamber and then through a pleated microporous fiberglass cartridge (Filterite) filter or through some other suitable filter. The viruses adsorb to the filter, and the water that passes through the filter is metered and discarded. The viruses are eluted from the filter with alkaline 3% beef extract or with some other suitable elutant, which is passed through the filter; the viruses are concentrated from the elutant by an organic flocculation technique (Katzenelson et al., 1976) or by some other suitable procedure. The concentrate is then assayed for viruses in appropriate cell cultures. With hepatitis A virus and with rotaviruses, special procedures are needed to detect and quantify the viruses.

With the aid of U.S. EPA funding support, the American Society for Testing Materials task group undertook a preliminary round-robin study directed at determining the accuracy and precision of a negatively charged virus-adsorbing filter procedure named the VIRADEL method. Six laboratories participated. A single poliovirus 1 stock, prepared in two concentrations, was seeded into 100 ℓ quantities of local tap waters at the participating laboratories and concentrated by the VIRADEL procedure and by any other procedure the participating laboratory wished to use. Recoveries for the low input seeding (350-860 PFU) were 8-20% in two laboratories, 49-63% in three laboratories, and 198% in ene laboratory. Recoveries for the high input seeding (1837-4689 PFU) were 6-12% in two laboratories and 26-46% in four laboratories. The task group recommended the procedure as a provisional method for recovering human enteroviruses from water (Melnick et al., 1984).

Positively Charged Virus-Adsorbing Filters. Microporous filters composed of charge-modified cellulose-diatomaceous earth-resin mixtures are now available that are positively charged at pH levels up to ~6. These filters adsorb about 99% of polioviruses from seeded tap water at pH levels up to ~7.5 with no salt added. In laboratory studies, these filters appear to be as efficient as the negatively charged microporous filters for recovering some viruses and are much simpler to use, because they require neither pH nor cation adjustment over a wide pH range (Sobsey and Glass, 1980). However, it seems likely that pH adjustment will be necessary at pH >8. Moreover, the degree to which humic acids and other organics interfere with virus recoveries on these filters is not yet known.

Deficiencies of Current Virus Recovery Methodology

The capsid proteins of two different strains of the same virus type may differ, the capsid proteins of two different virus types may differ even more, and the capsid proteins of viruses of different families may differ much more. As proteins differ, so do optimal conditions for adsorbing and eluting viruses to and from filters. Thus, enteroviruses adsorb to certain filters optimally at pH 3.5 (Berg et al., 1971a; Wallis et al., 1972), while adenoviruses adsorb best to the same filters at pH 5-6 (Fields and Metcalf, 1975). Optimal conditions for recovering rotaviruses (Smith and Gerba, 1981) and coliphages (Goyal et al., 1980) from large volumes of water also differ from those optimal for enteroviruses.

Experimentally, Gerba (1985) recovered rotaviruses more efficiently on negatively charged Filterite filters than on positively charged filters. Yet, he recovered rotaviruses on positively charged filters from 3/26 samples of drinking water in Mexico that had received full treatment. This drinking water contained 1 NTU or less of turbidity and had been exposed to at least 0.5 mg/ℓ of free chlorine for at least 30 minutes (Gerba, 1985). Gerba used positively charged filters because the use of negatively charged filters would have required carrying into the field heavy equipment necessary for acidifying and salting the waters.

Some bacterial viruses appear to be sensitive to the low pH (3.5) required for recovering enteroviruses on negatively charged Filterite filters. Thus, Goyal et al. (1980) obtained better recoveries of coliphages MS2, ϕX174, T2 and T4, seeded into tap waters and into other waters, on positively charged filters than on negatively charged Filterite filters. The ASTM (1983) published a proposed method for coliphages in tap waters, but the method is capable of recovering only those coliphages that multiply in the host bacterium used. In all, optimizing a recovery system that can serve for all or even many coliphages continues to be a problem.

The chemical quality of the water processed is probably one of the important factors that affects the capability of many virus recovery procedures. The humic acids and other organics in tap waters may block adsorptive sites on adsorbing filters and elute to waste viruses that are already adsorbed. Melnick et al. (1984) believed that the chemical qualities of the different tap waters they tested accounted in part for the variations in virus recoveries they encountered in the round-robin tests. They also attributed some of the variations encountered to failure to use a standardized eluting agent (beef extract) and standardized filters. A total standardization will probably be required for the method, as the task group prepares for a final round-robin series of tests.

Methods for Detecting Viruses in Drinking Water

Viruses in drinking water must be detected by a variety of methods, because there is no one detection system that can detect all viruses.

Most viruses multiply in cell cultures and produce easily recognizable cytopathology. These viruses can be detected and quantified by CPE in test tube cultures or by the plaque technique. Various groups of viruses and sometimes different viruses within a group grow best in different cells. For example, BGM cells are highly sensitive to many enteroviruses (Dahling et al., 1974), but others do not multiply in BGM cells. Certain group A coxsackieviruses do not multiply in BGM cells, but grow in RD cells (Schmidt et al., 1975, 1978) or in HFDK cells. Thus, to recover all enteroviruses in a sample, several cell lines must be used concurrently. If other viruses are to be recovered, additional cell lines may also be necessary. A list of commonly used cell culture systems sensitive to many of the viruses that may be present in environmental waters is presented in Table VIII-2.

Some viruses (such as Norwalk and Norwalk-like viruses and calici viruses) that probably occur in water have not been shown to grow in cells in culture and must be detected by immune EM or by other sophisticated techniques. Hepatitis A virus and astroviruses multiply in some cells, but do not produce cytopathic changes. Both viruses can be detected by immunoflourescence techniques (Smith and Gerba, 1982). Hepatitis A virus can also be detected by RIA or radioimmunofocus assay (Sobsey et al., 1984).

TABLE VIII-2

Commonly Used Cell Cultures for Propagating Human Enteric Viruses[a]

Cell Culture	Virus[b]							
	Adeno	Astro	Cox A	Cox B	Echo	Polio	Reo	Rota
Primary								
RhMK	+		–[c]	+	++	+	++	
AGMK					+	+	+	++[d]
HEK	++[e]	+[f]	+	+		+	+	+
Diploid or continuous								
MFK	–		–	+	+	+	+	
BGM	–		–	++	+	++	+	
BSC-1	–		–			+	+	
Vero							+	
MA-104							+	+
HFDK	+		++	–	+			
HEp-2 (HeLa)	+			+				
RD	–		++	–	++	++	–	
Graham 293	+[g]							

[a] Source: Modified from Smith and Gerba (1982)

[b] The caliciviruses and the Norwalk and Norwalk-like viruses were excluded from this table because they have not been propagated in cell cultures.

[c] Coxsackieviruses A9 and A16 replicate in primary monkey kidney cells.

[d] One strain of human type 2 rotavirus has been adapted to multiply in AGMK cells whereas rotavirus replication in the other cell cultures is determined by the presence of viral antigens.

[e] Adenoviruses 40 and 41 replication detected by immunofluorescence.

[f] Astrovirus replication detected by immunoflourescence.

[g] Adenoviruses 40 and 41 replicate in Graham 293 cells.

Cell cultures: RhMK, rhesus monkey kidney; AGMK, African green monkey kidney; HEK, human embryonic kidney; MFK, MA-104, fetal rhesus monkey kidney; BGM, Buffalo green monkey kidney; HFDK, human fetal diploid kidney; HEp-2, HeLa, human malignant epithelial cells; RD, human rhabdomyosarcoma.

(-), Virus replicates poorly or not at all in this cell culture; (+) virus replicates in this cell culture; (++), optimal cell culture for virus replication.

Methodology is not sufficiently developed for detecting Norwalk and Norwalk-like viruses, astroviruses, and some other fastidious viruses in drinking water. The detection of hepatitis A virus in drinking water has been recently achieved (Sobsey et al., 1984).

Immunofluorescence techniques may show high sensitivity, but 10^5 to 10^6 virus particles may be required before detection is possible by RIA or by enzyme assay techniques. EM techniques usually require the greatest number of virus particles (Smith and Gerba, 1982).

IX. POTENTIAL INDICATORS FOR WATERBORNE VIRUSES

Viruses have been recovered from chlorinated waters from which total coliform organisms could not be recovered (Petrilli et al., 1974; Berg and Metcalf, 1978; Melnick and Gerba, 1982). Thus, although total coliforms when present may be useful indicators of viruses, the absence of total coliforms from a drinking water, in standard tests, is no guarantee that viruses are absent. In this sense, total coliforms are a one way indicator; therefore, alternative indicators of viruses have been considered over the years.

The Ideal Indicator of Viruses in Drinking Water

The ideal indicator of viruses in a drinking water must always be present when the virus is present and absent when the virus is absent. The ideal indicator should derive from the feces as the virus does. The indicator must be present in the same numbers, or at least in a constant ratio (with the indicator present in greater numbers) to the viruses so that a count of the indicator organisms gives a good estimate of the numbers of viruses present. The ideal indicator must also be easily quantifiable. The ideal indicator and the virus must be about equally resistant to the environment, to treatment processes, and to disinfectants. An acceptable indicator of viruses in drinking water is one that is always present when viruses are present, is easily quantifiable, and is not more resistant than the virus to disinfection.

Logic in Choice of Indicator Systems

For many decades, total coliforms have been the indicator organisms that signaled the safety of drinking waters. However, many of the bacteria that constitute the total coliform group, however, are not of fecal origin; thus,total coliforms are an invalid indicator of fecal pollution. The fecal coliforms, which make up ~10% of the total coliforms in sewage, are fecal indicators and are, therefore, valid indicators of fecal pollution. Total coliforms include many nonfecal, lactose-fermenting, gram-negative bacilli and are present in sewage in numbers ~10-fold greater than the fecal coliforms. The bacteria that constitute the total coliform group are about equally resistant to disinfection. As an indicator system, therefore, total coliforms add a ~10-fold factor of safety when the system is used for a disinfected water. For a nondisinfected drinking water, however, an indicator system that includes so many organisms of nonfecal origin is not appropriate. Many groundwater supplies used for drinking are still not disinfected. Only an indicator consisting of fecal organisms is useful for such nondisinfected waters. Whether coliform organisms are completely adequate as indicators of viruses is debatable, since viruses have been recovered from nondisinfected and from disinfected waters from which neither fecal coliforms nor total coliforms were recovered. Viruses are more resistant than all coliforms to disinfection (Berg, 1978a).

Total Coliforms

Although total coliforms are a useful indicator of the safety of a disinfected drinking water (or of a disinfected swimming water), consistent total coliform:virus ratios do not occur in any waters (Morris and Waite, 1981; Shuval and Katzenelson, 1972; Allen and Geldreich, 1978; Berg and Metcalf, 1978). The reason for this is that total coliforms are ubiquitous in the water and soil environments, and enteric viruses of man are excreted only by infected individuals. The numbers of infected individuals within a community has no relationship to the ubiquitous presence of total coliforms in its receiving water.Stetler et al. (1984) reported total coliform:virus ratios ranging from 14:1 to >46,000:1 (CFU total coliforms/100 mℓ:PFU viruses/380 ℓ) in 11 samples of source and treated waters of a conventional water treatment plant in Michigan. Berg and Metcalf (1978) reported data on the New York bight that showed total coliform:virus ratios ranging from 66,000:1 to 1,700,000:1 (CFU total coliforms/100 mℓ:PFU viruses/100 mℓ). They also reported data on the Houston ship channel that showed total coliform:virus ratios ranging from 350,000:1 to 65,000,000:1 (CFU total coliforms/100 mℓ: PFU viruses/100 mℓ). Vaughn et al. (1978) found viruses in 6 of 30 well samples down gradient of basins charged with chlorinated effluents. The total coliform:virus ratios ranged from 1.4:1 to 18,000:1 (CFU total coliforms/100 mℓ:PFU viruses/3.8 ℓ), a range of ~4 orders of magnitude.

Craun (1978) lists several outbreaks of water transmitted viral diseases in which no total coliform organisms were recovered from the contaminated waters. Marzouk et al. (1979) found viruses in 20% of groundwater samples that were negative for total coliforms. Tyler (1983) recovered viruses from eight samples of raw water that contained 10 or less total coliforms per 100 mℓ of water and from 24 treated drinking waters only one of which contained total coliforms. Payment (1981), however, detected no total coliforms in conventionally-treated drinking waters from which he recovered as many as 100 viruses per 100 ℓ of water.

Alternative Indicator Systems

A number of organisms other than coliforms have been considered as alternative indicators for the safety of drinking water.

In a disinfected water, the indicator system can be an indicator of disinfection. In a nondisinfected drinking water, the indicator system must be an indicator of fecal pollution. The organisms that have been considered as alternatives to coliforms as indicators of viruses in drinking waters include the fecal streptococci, the total plate count (nonsporeformers), *Clostridium perfringens*, bifidobacteria, Bacteroides, yeasts, mycobacteria and bacteriophages.

Fecal Coliforms. Because almost all humans excrete fecal coliforms and because these organisms do not derive from nonfecal sources, fecal coliforms are valid indicators of human and other animal pollution. The presence of fecal coliforms in drinking or recreational waters validly forewarns of the possible presence of viruses. However, the absence of such organisms does not guarantee the absence of viruses. Moreover, the ratios of fecal coliform to human viruses in environmental waters are not constant because almost all persons excrete fecal coliforms, but only infected individuals excrete viruses.

Thus, Berg and Metcalf (1978) reported fecal coliform:virus ratios ranging from 14,000:1 to 7,600,000:1 in the Missouri River and ratios ranging from 33,000:1 to 7,500,000:1 in the Mississippi River. Many of the samples that contained viruses were collected at or near water intakes. In the New York bight, fecal coliform:virus ratios ranging from 4300:1 to 350,000:1 were reported. In the Houston ship channel, fecal coliform:virus ratios ranged from 110,000:1 to 28,000,000:1 (Berg and Metcalf, 1978).

In a study on raw waters and drinking waters, Tyler (1983) found viruses in 52 samples, 11 of which contained 10 or fewer E. coli per 100 mℓ of water (E. coli is the most common of the fecal coliform organisms). Tyler also recovered viruses from 24 samples of chlorinated drinking waters, only one of which contained E. coli in a 100 mℓ sample.

Vegetative Bacteria (Total Counts). If total coliform counts are useful as indicators of the safety of a disinfected drinking water because the numbers of total coliforms in environmental waters are considerably greater than the numbers of fecal coliforms, then a total count of all vegetative bacteria should present an even greater margin of safety for a disinfected drinking water. With a nondisinfected drinking water total plate counts of vegetative bacteria are no more useful than total coliform counts, because most of the organisms counted in a vegetative cell count have no more sanitary significance than most of the organisms in a total coliform count have. Tyler (1983), for example, found numbers in 22°C plate counts ranging from 7-44,800 CFU/100 mℓ in 12 raw water samples that contained from 0.83-15 PFU of viruses/10 ℓ of water. There was no correlation between the numbers of viruses in the waters and the 22°C plate counts. In chlorinated drinking waters, Tyler (1983) found 22°C plate counts ranging from 1-21 CFU/100 mℓ of drinking waters that contained from 0.63-6 PFU of viruses/10 ℓ of the water.

It is possible that some of the vegetative bacteria found in such counts may be natural slime producers or may become encased in the slime that develops in the walls of pipes that carry potable waters. These organisms may be protected from chlorination and thereby bear false witness against a safe water supply. Chromogenic bacteria have been recovered from chlorinated waters in drinking fountains in Cincinnati, OH. Once grown in cultures and freed of slime, these organisms were no more resistant than coliforms to chlorination (Berg and Kenner, unpublished data). Nonetheless, a vegetative bacterial indicator for chlorinated drinking water warrants investigation.

Fecal Streptococci. The fecal streptococci are probably the most viable alternative indicator of fecal pollution available. Fecal streptococci with the possible exception of Streptococcus faecalis var liquifaciens occur only in the intestines of warm-blooded animals. Fecal streptococci occur in lesser numbers than fecal coliforms in the feces of man, but in larger numbers than fecal coliforms in the intestines of certain animal species. These differences led to the use of fecal coliform to fecal streptococcus ratios for indicating whether a pollution source is composed of human or animal wastes (Geldreich, 1978).

In seeded experiments, Streptococcus faecalis, the predominant fecal streptococcus in the feces of humans, survived longer in the tap waters of 13 different U.S. cities than E. coli, the predominant fecal coliform in the feces of humans and other warm-blooded animals, Enterobacter aerogenes, and two human intestinal pathogens, Salmonella typhimurium, and Shigella sonnei (Table IX-1) (Kenner, 1978). Moreover, S. faecalis did not multiply in these tap waters. The coliforms and salmonella multiplied in some of them.

Fecal streptococci persist longer in many waters than fecal coliforms do (Kenner, 1978). Furthermore, because they are clumped, fecal streptococci

TABLE IX-1

Persistence of Indicator Organisms and Pathogenic Bacteria in Municipal Drinking Water at 20°C*

Organism	Day	[illegible], Alabama	[illegible], Alabama	Pensacola, Florida	Lexington, Kentucky	New Orleans, Louisiana	Louisville, Kentucky	[illegible], Texas	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		Survival of Organisms (%)												
Escherichia coli	1	6	20	2	100	38	56	74	68	63	8	49	24	54
	2	13	5	0	1160	138	144	256	68	90	0	11	1	3
	3	5	1	0				GR	93					3
	7				GR	GR	GR			GR	0	0	0	
Enterobacter aerogenes	1	43	17	23	119	290	104	106	70	79	60	38	21	62
	2	9	4	36	330	GR	GR	21	480	117	32	29	3	46
	3	5	1	11				2	GR					27
	7				GR	GR	GR			GR	GR	GR	0	
Streptococcus faecalis	1	86	59	83	90	89	88	78	61	129	71	50	61	100
	2	41	46	54	70	89	88	65	61	129	58	83	78	67
	3	36	55	54				59	57					58
	7				80	50	63			93	58	17	28	
Salmonella typhimurium	1	58	50	50	170	119	42	83	90	120	0	40	100	113
	2	25	5	237	GR	440	6	71	130	230	0	19	58	240
	3	20	0	365				79	148					GR
	7				GR	GR	8			GR	0	0	53	
Shigella sonner	1	8	19	6	45	59	20	29	71	73	30	44	47	37
	2	10	30	6	15	12	3	10	24	31	15	9	32	5
	3	10	0	42				3	7					5
	7				0	4	0			4	0	0	7	

*Source: Abstracted and revised from Kenner (1978)

GR: Growth of bacteria

may survive in chlorinated waters after all fecal coliforms and total coliforms have been inactivated (Cohen and Shuval, 1973; Berg, unpublished data).

Tyler (1983) recovered a fecal streptococcus from 8 of 9 chlorinated drinking water samples that contained viruses. Only one of the samples that contained a fecal streptococcus also contained total coliforms.

In digested sludges, fecal streptococci dieoff at the same rate as enteroviruses. The dieoff rates of total coliforms and fecal coliforms are not correlated (Berg and Metcalf, 1978).

Recently, Cabelli (1983) and Dufour (1984) reported a correlation between enterococci, a subset of the fecal streptococci, in recreational waters and the occurrence of GI symptoms in recreational bathers. However, they reported an incidence of GI symptoms three times higher in marine water bathers than in fresh water bathers for the same levels of enterococci in the water. Cabelli and Dufour found similar correlations for *Escherichia coli* but not for fecal coliforms.

The fecal streptococci indicator system is finding new proponents as shortcomings of the coliform indicator systems become more evident.

Clostridium Perfringens. *Clostridium perfringens* is an anaerobic spore-forming constituent of normal fecal flora, and is present in feces in large numbers. Its relative resistance to chlorine and other disinfectants offers a large margin of safety for disinfected drinking waters when their microbiological safety is judged by the absence of these clostridia.

Although viruses are considerably more resistant to disinfectants than most vegetative bacteria, viruses are not as resistant as bacterial spores (Berg, 1978a). Some European and American researchers have long advocated C. perfringens as an indicator of fecal pollution in chlorinated and other waters and especially for detection of remote pollution (Bonde, 1963; Cabelli, 1977, 1978; Goyal, 1983). However, the relative resistance of C. perfringens to disinfection could easily result in condemnation of microbiologically safe disinfected waters, and the long persistence of C. perfringens in environmental waters could bring about the condemnation of microbiologically safe nondisinfected drinking waters. Moreover, the methods for detecting this anaerobe are not as simple and inexpensive as one would need for a routine indicator system.

Bifidobacteria. Bifidobacteria are present in large numbers in the feces of warm-blooded animals. These bacteria appear not to multiply in the water environment, and studies have suggested that they survive in nature about as well as fecal coliforms and intestinal pathogens. Thus, a suggestion was made that the bifidobacteria should be considered an alternative fecal indicator (Evison and James, 1975; Levin, 1977). Bifidobacteria are present in fecal material in larger numbers than are fecal coliforms. The resistance of bifidobacteria to water disinfectants still requires study, and the methods for routine detection of this obligate anaerobe are not as simple and inexpensive as the methods for currently available indicator systems.

Mycobacteria and Yeasts. Acid-fast organisms and yeasts were recovered from chlorinated sewage during a search for chlorine-resistant organisms that might serve as indicators of viruses in chlorinated effluents (Engelbrecht and Greening, 1978). The yeast recovered was identified as _Candida parapsilosis_ and the acid-fasts were identified as _Mycobacterium fortuitum_ and _Mycobacterium phlei_. All three organisms were more resistant than enteroviruses to chlorine. The yeast and _M. phlei_ were ~20 times more resistant to chlorine than the enteroviruses tested and the _M. fortuitum_ was ~5 times more resistant than the _M. phlei_ and the _Candida_. However, the methods for growing and demonstrating these organisms do not yield rapid results, and their natural habitats are not yet well defined. Whether these organisms occur in drinking waters is not clear, either. The possible use of these organisms as indicators in drinking waters would seem to be of some interest at this time.

Bacterial Viruses (Bacteriophages). Although only the coliphages among the bacteriophages have been considered as possible indicators for the microbiological safety of disinfected drinking waters, any ubiquitous phage might be used. The total coliform indicator itself, has little sanitary significance. Total coliforms are used because they are present in much greater numbers than are the fecal coliforms. The margin of safety advantage of phages is that, like most other viruses, they tend to be more resistant to disinfection than vegetative bacteria. For drinking waters that are not disinfected, phages not derived from fecal coliforms are of no more sanitary significance than total coliforms.

Kott el al. (1974) found that coliphages MS2 and f2 survived as long as poliovirus 1 in an experimental oxidation pond. Also, these phages were as resistant to chlorination as the poliovirus. These findings led Kott and his colleagues to suggest that coliphages may better signal the microbiological safety of waters than coliforms. Moreover, coliphage assays are simple to do, yield rapid results, and are relatively inexpensive.

However, in an extensive 3-year study in an estuary, Vaughn and Metcalf (1975) frequently recovered coliphages from samples that yielded no enteroviruses and frequently recovered enteroviruses from samples that yielded no coliphages. Vaughn and Metcalf (1975) used three different _E_. _coli_ host strains, _E_. _coli_ 11303, _E_. _coli_ 9637 and _E_. _coli_ UNH. Strain 11303 yielded 3 times as many coliphage isolates as strain 9637 and 6 times as many as strain UNH during the first year of the study. During the last 2 years of the study, however, strain 11303 yielded many fewer coliphage isolates than strain 9637. Vaughn and Metcalf's findings demonstrated that coliphage recovery depended upon using hosts sensitive to the coliphage strains present in the water. However, there was no way to predict which hosts would be most sensitive at any given time.

Simkova and Cervenka (1981) reported that enteric viruses were recovered from the Danube River when coliphage counts were high (during the colder months) but not when coliphage counts were low (during the summer months).

Average coliphage counts (on _E_. _coli_ _C_) at a water plant intake in Michigan outnumbered average enteric virus counts by > 23,000:1, and the number of coliphages and the number of enteric viruses in these waters fluctuated in parallel (Stetler, 1984) . Thus, Stetler concluded, coliphages may be a useful indicator of enteric viruses in drinking waters. Scarpino (1978) commented "The relative numbers of coliphages and enteric viruses in environmental waters, and the fluctuations of their numbers in these waters ... must be thoroughly documented before either one or an array of phages can be promulgated as indicators of enteric viruses or bacteria."

The distribution of bacteriophages in sewage was studied. Dhillon and Dhillon (1974) reported that *E. coli* strains newly isolated from natural sources were inadequate hosts for coliphages in sewage, but lac$^-$ mutants of *E. coli* infected with a sex factor of *E. coli* strain K-12 were excellent hosts for F-specific coliphages in sewage. Most of the RNA phages recovered on this host were related to MS2 and many others were related to QB phages. In most habitats, only one of the phages was found. Single-stranded DNA F-specific phages were not detected with their techniques. Dhillon et al. (1970) surveyed coliphages in Hong Kong sewage and found that half of those studied multiplied in *E. coli* strain K-12 and in *E. coli* B cells. About 32% were male specific and the rest were K-12 specific but sex indifferent. Ignazzitto et al. (1980) compared the sensitivities of 10 strains of *E. coli* to phages in sewage and found a strain designated *E. coli* 8113 to be the best among those tested. They concluded that in sewages stored for 2 weeks, coliphages were better indicators than their coliform hosts.

Furuse et al. (1979b) classified RNA coliphages into four groups (Groups I to IV), based on physicochemical and serological properties. Domestic sewage in Japan contained Groups II and III RNA coliphages, which constituted the predominant phage type (Furuse et al., 1978, 1979a, 1981). The sewage from slaughterhouses and from animal feces contained Group I coliphages only. Human excreta contained almost equal numbers of Groups II and III coliphages. The frequency of isolation of these phages from humans and animals was only 2 and 6%, respectively. Although Groups II and III coliphages were also recovered from sewages in the United States,sewage from Mexico, Saudi Arabia and Brazil contained none of these groups and sewages from West Germany and the Phillipines contained only one or the other of these groups (Furuse et al., 1975, 1978). These data seem to corroborate the data of Vaughn and Metcalf (1975), which imply a lack of long continuity of phage types in any one area.

Primrose et al. (1981) divided coliphages into three physiological types depending upon their efficiency of plating at different temperatures: HT coliphages, plated at 25°C or higher, LT coliphages plated at or below 30°C, and mid-temperature phages, plated in the range of 15-42°C. The distribution of the three types of phages correlated well with the temperature of the environment from which they were recovered. The HT type predominated in sewage effluents implying a possible indicator value for such coliphages.

Coliphages offer some advantages over total coliforms as indicators for the microbiological safety of disinfected drinking waters. They are more resistant than total coliforms to disinfection, and the time required for a coliphage assay is less than required for a total coliform assay. However, before a coliphage indicator is used as an alternative to the total coliform indicator for the microbiological safety of a disinfected drinking water, extensive surveys will be required to demonstrate that coliphages are detected in drinking water whenever total coliforms are detected. Before a coliphage indicator can be used as an indicator of viruses in disinfected drinking water, similar surveys will be required to demontrate that coliphages are detected in drinking water whenever viruses are detected and at least in equal numbers.

For nondisinfected drinking waters, coliphages can only be useful as indicators if the coliphages multiply only in fecal coliforms.

Conclusions

Total coliforms are a useful one-way indicator for enteric viruses in disinfected drinking waters. In disinfected drinking water, the presence of total coliforms denotes inadequate disinfection. In nondisinfected drinking water total coliforms are of little value as an indicator system. Since viruses have been recovered from disinfected waters in the absence of total coliforms, the absence of total coliforms in such waters cannot give absolute assurance that viruses are also absent.

The virus itself is its own best possible indicator. The suggestion that some viruses infectious to man serve as indicators of other viruses that infect man is valid, but the presence of any such virus constitutes the presence of the pathogen itself. No additional indicator is necessary.

Although a number of alternative indicator systems for human viruses have been considered, data to support their acceptance are sparse. Fecal streptococci appear to be more resistant than total coliforms to the environment and to disinfection, but are present in feces, and thereby in domestic sewage, in much smaller numbers than are the total coliforms. Moreover, few data correlate the presence of fecal streptococci with viruses in drinking waters. The same problem exists for other alternative indicator candidates: Clostridium perfringens, bifidobacteria, mycobacteria and yeasts.

The bacterial viruses, and especially the coliphages, seem to excite most interest as alternative indicators, because like most viruses, phages tend to be more resistant to disinfection than most bacterial indicators. Also, phages are easy and inexpensive to grow, and are ubiquitous in the water environment. There are limited indications that some coliphages may be feces-specific. However, there are few data available showing the presence of coliphages in drinking waters that contain enteric viruses, and there are no data available correlating the presence of one with the other in drinking water. The collection of appropriate data would be most useful.

X. REFERENCES

Aach, R.D. 1976. Viral Hepatitis - Update. Viewpoints Dig. Dis. 8: 1.

Akin, E.W. 1977. Viruses in finished water: The occoquan experience. Proc. Am. Water Works Assoc. WQTC, Denver, CO.

Akin, E.W. 1983. A review of infective dose data for enteroviruses and other enteric microorganisms in human subjects. In: Microbial Health Considerations of Soil Disposal of Domestic Wastewaters: Proceedings of a Conference, May 11-12, 1982, University of Oklahoma, Norman, OK. EPA 600/9-83-017, September, 1983.

Akin, E.W., W.H. Benton and W.F. Hill, Jr. 1971. Enteric viruses in ground and surface waters: A review of their occurrence and survival. In: Virus and Water Quality: Occurrence and Control, V. Snoeyink, Ed. Proc. 13th Water Quality Conf., University of Illinois, Urbana, IL.

Allen, M.J. and E.E. Geldreich, Jr. 1978. Evaluating the microbial quality of potable waters. In: Evaluation of the Microbiology Standards for Drinking Water, C.W. Hendricks, Ed. EPA 570/9-78-002.

American Water Works Association Committee on Viruses in Drinking Water. 1979. Viruses in water. J. Am. Water Works Assoc. 71: 441.

APHA (American Public Health Association). 1980. Standard Methods for the Examination of Water and Wastewater, 15th ed. American Public Health Association, Washington, DC.

Armstrong, C. 1939. The experimental transmission of poliomyelitis to the eastern cotton-rat. Pub. Health Rep. 54: 1719.

ASTM (American Society for Testing Materials). 1983. Standard Test Method for Coliphages in Water, Designation D4201-82, American Society for Testing Materials, Philadelphia, PA.

Bancroft, P.M., W.E. Engelhart and C.A. Evans. 1957. Poliomyelitis in Huskerville (Lincoln) Nebraska; Studies indicating a relationship between clinically severe infections and proximate fecal pollution of water. J. Am. Med. Assoc. 164: 836.

Beard, J.W. 1967. Host-virus interaction in the initation of infection. In: Transmission of Viruses by the Water Route, G. Berg, Ed. John Wiley & Sons, New York.

Belshe, R.B., Ed. 1984. Textbook of Human Virology. Enteroviruses, Including Polioviruses. p. 401-477.

Berg, G. n.d. Unpublished data.

Berg, G. 1971. Integrated approach to problem of viruses in water. J. Sanit. Eng. Div., Proc. Am. Soc. Civil Eng. 97: SA6:867.

Berg, G. 1973a. Reassessment of the virus problem in sewage and in surface and renovated water. In: Progress in Water Technology, Vol. 3, S.H. Jenkins, Ed. Pergamon Press, Elmsford, NY.

Berg, G. 1973b. Removal of Viruses From Sewage, Effluents, and Waters. I. A Review. Bull. WHO. 49: 451.

Berg, G. 1978a. The indicator system. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Berg, G. 1978b. Viruses in the environment: Criteria for risk. In: Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, B.P. Sagik and C.A. Sorber, Ed. The Center for Applied Research and Technology, The University of Texas at San Antonio.

Berg, G. 1984. Monitoring for viruses disseminated in water. Monogr. Virol. 15: (in press).

Berg, G. and B.A. Kenner. n.d. Unpublished data.

Berg, G. and T.G. Metcalf. 1978. Indicators of viruses in waters. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Berg, G., D. Berman and D.R. Dahling. n.d. Unpublished data.

Berg, G., D.R. Dahling and D. Berman. 1971a. Recovery of small quantities of viruses from clean waters on cellulose nitrate membrane filters. Appl. Microbiol. 22: 608.

Berg, G., D.R. Dahling, D. Berman and C. Walter. 1971b. The virus hazard on the Missouri River. In: Everyone Can't Live Upstream, Region 7, U.S. EPA, Kansas City, MO.

Berg, G., D.R. Dahling, G.A. Brown and D. Berman. 1978. Validity of fecal coliforms, total coliforms, and fecal streptococci as indicators of viruses in chlorinated primary sewage effluents. Appl. Environ. Microbiol. 36: 880.

Berg, G., R.S. Safferman, D.R. Dahling, D. Berman and C. Hurst. 1984. Chapter 1, U.S. EPA Manual of Methods for Virology, Environmental Monitoring and Support Laboratory, Cincinnati, OH. (In press)

Berry, J.K. and P.T.B. Shaffer. 1978. Viruses: Monitoring is the key. Water Wastes Eng. 15: 15.

Bitton, G. 1978. Survival of enteric viruses. In: Water Pollution Microbiology, Vol. 2, R. Mitchell, Ed. John Wiley & Sons, New York.

Bitton, G. and R. Mitchell. 1974. Effect of colloids on the survival of bacteriophages in seawater. Water Res. 8: 227.

Blacklow, N.R., G. Cukor, M.K. Bedigian, et al. 1979. Immune response and prevalence of antibody to Norwalk enteritis virus as determined by radioimmunoassay. J. Clin. Microbiol. 10: 903.

Block, J.C. 1983. Viruses in environmental waters. In: Viral Pollution of the Environment, G. Berg, Ed. CRC Publishers Inc., Boca Raton, FL.

Block, J.C., J.C. Joret, M. Morlot and J.M. Foliguet. 1978. Recherche des Enterovirus dans les Eaux Superficielles par Adsorption-e'lution sur Microfibre de Verre. Tech. Sci. Munic.-l'Eau. 73: 181. (Fre.)

Block, J.C., J.F. Collin, J.C. Joret, D. Rolland and J.M. Foliguet. 1979. Elimination des Enterovirus au Cours du Traitement par Boues Active'es des Eaux Use'es Urbaines. Tech. Sci. Munic.,-l'Eau. 74: 201. (Fre.)

Bodian, D. 1955. Emerging concept of poliomyelitis infection. Science. 122: 105.

Bodian, D. 1956. Poliovirus in chimpanzee tissues after virus feeding. Am. J. Hyg. 64: 181.

Bodian, D., I.M. Morgan and H.A. Howe. 1949. Differentiation of types of poliomyelitis viruses. III. The grouping of fourteen strains into three basic immunological types. Am. J. Hyg. 49: 234.

Bolivar, R., R.H. Conklin, J.J. Vollet, et al. 1978. Rotavirus in traveler's diarrhea: Study of an adult student population in Mexico. J. Infect. Dis. 137: 324.

Bonde, G.J. 1963. Bacterial Indicators of Water Pollution, Teknisk. Forlag, Copenhagen.

Boyer, G.S., C. Leuchtenberger and H.S. Ginsberg. 1957. Cytological and cytochemical studies of HeLa cells infected with adenoviruses. J. Exper. Med. 105: 192.

Brandt, C.D., H.W. Kim, W.J. Rodriguez, et al. 1983. Pediatric viral gastroenteritis during eight years of study. J. Clin. Microbiol. 18: 71.

Buras, N. 1976. Concentration of enteric viruses in wastewater effluent: A two-year survey. Water Res. 10: 295.

Cabelli, V.J. 1977. Clostridium perfringens as a water quality indicator. In: Bacterial Indicators/Health Hazards Associated with Water, A.W. Hoadley and B.J. Dutka, Ed. ASTM Special Tech. Publ. No. 635, American Society for Testing Materials, Philadelphia, PA.

Cabelli, V.J. 1978. Obligate anaerobic bacterial indicators. In: Viral Pollution of the Environment, G. Berg, Ed. CRC Press Inc., Boca Raton, FL.

Cabelli, V.J. 1983. Health Effects Criteria for Marine Recreational Waters. Health Effects Research Laboratory, U.S. EPA, Cincinnati, OH. EPA 600/1-80-031.

Cabelli, V.J., A.P. Dufour, L.J. McCabe and M.A. Levin. 1982. Swimming associated gastroenteritis and water quality. Am. J. Epidemiol. 115: 606.

CDC (Centers for Disease Control). 1974. Foodborne and Waterborne Disease Outbreaks, Annual Summary 1973. U.S. DHEW, Atlanta, GA.

CDC (Centers for Disease Control). 1975. Foodborne and Waterborne Disease Outbreaks, Annual Summary 1974. U.S. DHEW, Atlanta, GA.

CDC (Centers for Disease Control). 1976. Foodborne and Waterborne Disease Outbreaks, Annual Summary 1975. U.S. DHEW, Atlanta, GA.

CDC (Centers for Disease Control). 1977. Foodborne and Waterborne Disease Outbreaks, Annual Summary 1976. U.S. DHEW, Atlanta, GA.

CDC (Centers for Disease Control). 1979. Foodborne and Waterborne Disease Surveillence, Annual Summary 1977. U.S. DHEW, Atlanta, GA.

CDC (Centers for Disease Control). 1980. Water-related Disease Outbreaks Surveillance, Annual Summary 1978. U.S. DHHS, Atlanta, GA.

CDC (Centers for Disease Control). 1981. Water-related Disease Outbreaks Surveillance, Annual Summary 1979. U.S. DHHS, Atlanta, GA.

CDC (Centers for Disease Control). 1982a. Water-related Disease Outbreaks Surveillance, Annual Summary 1980. U.S. DHHS, Atlanta, GA.

CDC (Centers for Disease Control). 1982b. Water-related Disease Outbreaks Surveillance, Annual Summary 1981. U.S. DHHS, Atlanta, GA.

CDC (Centers for Disease Control). 1983. Water-related Disease Outbreaks Surveillance, Annual Summary 1982. U.S. DHHS, Atlanta, GA.

Chang, S.L., G. Berg, N.A. Clark and P.W. Kabler. 1960. Survival and protection against chlorination of human enteric pathogens in free-living nematodes isolated from water supplies. Am. J. Trop. Med. Hyg. 9: 136.

Chany, C., P. Le'pine, M. Lelong, V. Le Tan, P. Satge' and J. Virat. 1958. Severe and fatal pneumonia in infants and young children associated with adenovirus infection. Am. J. Hyg. 67: 367.

Clarke, N.A., E.W. Akin, O.C. Liu, et al. 1975. Virus study for drinking water supplies. J. Am. Water Works Assoc. 67: 192.

Cliver, D.O. 1967. Enterovirus detection by membrane chromatography. In: Transmission of Viruses by the Water Route, G. Berg, Ed. John Wiley & Sons, New York.

Cohen, J. and H.I. Shuval. 1973. Coliforms, fecal coliforms and fecal streptococci as indicators of water pollution. Water, Air, Soil Pollut. 2: 85.

Coin, L., M.L. Menetrier, J. LaBonde and M.C. Hannoun. 1966. Modern microbiological and virological aspects of water pollution. In: Advances in Water Pollution Research, O. Jeep, Ed. Pergamon Press, London. p. 1-10.

Conference on Monitoring for Viruses in the Environment. 1981. U.S. EPA Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Cook, M.K. and R.M. Chanock. 1963. In vivo antigenic studies of parainfluenza viruses. Am. J. Hyg. 77: 150.

Craighead, J.E., M.K. Cook and R.M. Chanock. 1960. Infection of hamsters with parainfluenza 3 virus. Proc. Soc. Expt. Biol. Med. 104: 301.

Craun, G.F. 1978. Impact of the coliform standard on the transmission of disease. In: Evaluation of the Microbiology Standards for Drinking Water, C.W. Hendricks, Ed. EPA 570/9-78-002, Washington, DC.

Craun, G.F. 1979. Waterborne disease outbreaks in the United States. J. Environ. Health. 41: 259.

Craun, G. 1981. Outbreaks of waterborne disease in the United States: 1971-1978. J. Am. Water Works Assoc. 70: 360.

Cubitt, W.D. and A.D.T. Barrett. 1984. Propagation of human candidate calicivirus in cell culture. J. Gen. Virol. 65: 1123.

Cukor, G. and N.R. Blacklow. 1984. Human gastroenteritis. Microbiol. Rev. Vol. 48(2): 157-179.

Dahling, D.R. and R.S. Safferman. 1979. Survival of enteric viruses under natural conditions in a subarctic river. Appl. Environ. Microbiol. 38: 1103.

Dahling, D.R., G. Berg and D. Berman. 1974. A continuous cell line more sensitive than primary rhesus and African green kidney cells for the recovery of viruses from water. Health Lab. Sci. 11: 275.

Dalldorf, G. 1957. Neuropathogenicity of certain group A coxsackieviruses. J. Exp. Med. 106: 69.

Davidson, G.P., I. Galler and R.F. Bishop. 1975. Immunoflourescence in duodenal mucosa of children with acute enteritis due to a new virus. J. Clin. Pathol. 28: 263.

Deetz, T.R., E.M. Smith, S.M. Goyal, et al. 1984. Occurrence of rota- and enteroviruses in drinking and environmental water in a developing nation. Water Research. 18: 567.

DeFlora, S., G.P. DiRenzi and B. Badolati. 1975. Detection of animal viruses in coastal sea water and sediments. Appl. Microbiol. 30: 472.

deJong, J.C., R. Wigand, A.H. Kidd, et al. 1983. Candidate adenoviruses 40 and 41: Fastidious adenoviruses from human infant stool. J. Med. Virol. 11: 215.

Dhillon, E.K.S. and T.S. Dhillon. 1974. Synthesis of indicator strains and density of ribonucleic acid-containing coliphages in Hong Kong sewage. Appl. Microbiol. 27: 640.

Dhillon, T.S., Y.S. Chan, S.M. Sun and W.S. Chau. 1970. Distribution of coliphages in Hong Kong sewage. Appl. Microbiol. 20: 187.

Dienhardt, F. and I.D. Gust. 1982. Viral Hepatitis. Bull. WHO. 60: 661.

Dimitrov, D.H., M.K. Estes, S.M. Rangelova, L.M. Shindarov, J.L. Melnick and K.Y. Graham. 1983. Detection of antigenically distinct rotaviruses from infants. Inf. Immunol. 41: 523.

Dingle, J.H., G.F. Badger, A.E. Feller, R.G. Hodges, W.S. Jordan, Jr. and C.H. Rammelkamp, Jr. 1953. A study of illness in a group of Cleveland families. I. Plan of study and certain general observations. Am. J. Hyg. 58: 16.

Drouhet, V. 1955. E'tude Comparative de la Sensibilite' des Cultures de Tissu Re'nal de Cercopithecus aethiops sabaeus et de Macacus phillipinensis aux Virus Poliomye'litiques et aux Autres Virus Enter'iques Humains. Ann. Inst. Pasteur. 89: 666. (Fre.)

Dufour, A.P. 1984. Health Effects Criteria for Fresh Recreational Waters. Health Effects Research Laboratory, Cincinnati, OH. EPA 600/1-84-004.

Echeverria, P., N.R. Blacklow, L.B. Sanford and G.G. Cukor. 1981. Travellers' diarrhea among American Peace Corps volunteers in rural Thailand. J. Infect. Dis. 143: 767.

Engelbrecht, R.S. and E.O. Greening. 1978. Chlorine-resistant indicators. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Evison, M. and A. James. 1975. Bifidobacterium as an indicator of fecal pollution in water. Prog. Water Technol. 7: 57.

Farrah, S.R., C.P. Gerba, C. Wallis and J.L. Melnick. 1976. Concentrate of viruses from large volumes of tapwater using pleated membrane filters. Appl. Environ. Microbiol. 31: 221.

Farrah, S.R., S.M. Goyal, C.P. Gerba, R.H. Conklin and E.M. Smith. 1978. Comparison between adsorption of poliovirus and rotavirus by aluminum hydroxide and activated sludge flocs. Appl. Environ. Microbiol. 35: 360.

Fields, H.A. and T.G. Metcalf. 1975. Concentration of adenovirus from seawater. Water Res. 9: 357.

Flores, J., R.H. Purcell, I. Perez, et al. 1983. A dot hybridisation assay for detection of rotavirus. Lancet. 1: 555.

Foliguet, J.M., L. Schwartzbrod and O.G. Gaudin. 1976. La Pollution Virale des Eaux Use'es, de Surface et d'Alimentation. Bull. WHO. 35: 737. (Fre.)

Fri, R.W. and J.H. McDermott. 1972. Hearings Before the Committee on Commerce, U.S. Senate, 92nd Congress, Serial 92-57, March 20, 1972, U.S. Gov't Printing Office, Washington, DC.

Furuse, K., A. Ando and I. Watanaba. 1975. Isolation and grouping of RNA phages. VII. A survey in Peru, Bolivia, Mexico, Kuwait, France, Australia, and the United States of America. J. Keio Med. Soc. 52: 355.

Furuse, K., T. Sakurai, A. Hirashima, M. Katsuki, A. Ando and I. Watanabe. 1978. Distribution of ribonucleic acid coliphages in South and East Asia. Appl. Environ. Microbiol. 35: 995.

Furuse, K., A. Hirashima, H. Harigai, et al. 1979a. Grouping of RNA coliphages based on analysis of sizes of their RNAs and proteins. Virology. 97: 328.

Furuse, K., A. Ando, S. Osawa and I. Watanabe. 1979b. Continuous survey of the distribution of RNA coliphages in Japan. Microbiol. Immunol. 23: 867.

Furuse, K., A. Ando, S. Osawa and I. Watanabe. 1981. Distribution of ribonucleic acid coliphages in raw sewage from treatment plants in Japan. Appl. Environ. Microbiol. 41: 1139.

Gamble, D.R. 1979. Viruses in drinking water. Reconsideration of evidence for postulated health hazard and proposals for virological standards of purity. Lancet. February 24, 425.

Geldreich, E.E. 1978. Bacterial populations and indicator concepts in feces, sewage, stormwater and solid wastes. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Gerba, C.P. 1980. Virus survial in wastewater treatment. In: Viruses and Wastewater Treatment, M. Goddard and M. Butler, Ed. Pergamon Press, New York. p. 39.

Gerba, C.P. 1983. Methods for recovering viruses from the water environment. In: Viral Pollution of the Environment, G. Berg, Ed. CRC Press Inc., Boca Raton, FL.

Gerba, C.P. 1984. Personal communication.

Gerba, C.P. and G.E. Schaiberger. 1975. The effect of particulates on virus survival in seawater. J. Water Pollut. Control Fed. 9: 567.

Gerba, C.P., S.N. Singh and J.B. Rose. 1985. Waterborne Gastroenteritis and Viral Hepatitis. CRC C.R. Environ. 15(3): 213-236.

Gifford, R. and G. Dalldorf. 1951. The morbid anatomy of experimental coxsackievirus infection. Am. J. Pathol. 27: 1047.

Girardi, A.J., K. Hummeler and I. Olshin. 1957. Studies on the routine laboratory diagnosis of coxsackie group B virus infections. I. The application of tissue culture procedures. J. Lab. Clin. Med. 50: 526.

Goldfield, M. 1976. Epidemiological indicators for transmission of viruses in water. In: Viruses in Water, G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, Ed. American Public Health Association, Washington.

Goyal, S.M. 1983. Indicators of viruses. In: Viral Pollution of the Environment, G. Berg, Ed. CRC Press, Inc., Boca Raton, FL.

Goyal, S.M. and C.P. Gerba. 1979. Comparative adsorption of human enteroviruses, simian rotavirus and selected bacteriophages to soils. Appl. Environ. Microbiol. 38: 241.

Goyal, S.M., C.P. Gerba and J.L. Melnick. 1978. Prevalence of human enteric viruses in coastal canal communities. J. Water Pollut. Control Fed. 50: 2247.

Goyal, S.M., K.S. Zerda and C.P. Gerba. 1980. Concentration of coliphages from large volumes of water and wastewater. Appl. Environ. Microbiol. 39: 85.

Grabow, W.O.K. and M. Isaacson. 1978. Microbiological quality and epidemiological aspects of reclaimed water. Prof. Water Technol. 10: 1.

Grabow, W.O.K., V. Gaussmuller, O.W. Prozesky and F. Deinhardt. 1983. Inactivation of hepatitis A virus and indicator organisms in water by free chlorine residuals. Appl. Environ. Microbiol. 46: 619.

Greenberg, H.B., J.R. Valdesuso, A.R. Kalica, et al. 1981. Proteins of Norwalk virus. J. Virol. 37: 994.

Haas, C.N. 1983. Estimation of risk due to low doses of microorganisms. A comparison of alternative methodologies. Am. J. Epidemiol. 118: 573.

Haley, C.E., R.A. Gunn, J.M. Hughes, E.C. Lippy and G.F. Craun. 1980. Outbreaks of waterborne disease in the United States, 1978. J. Infect. Dis. 141: 794.

Halvorsrud, J. and I. Orstavik. 1980. An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. Scand. J. Infect. 12: 161.

Hamilton, J.R. and D.G. Gall. 1982. Pathophysiological and clinical features of viral enteritis. In: Virus Infections of the Gastrointestinal Tract, D.A.J. Tyrell and A.Z. Kapikian, Ed. Marcel Dekker, Inc., New York.

Hannoun, C. 1965. Personal communication.

Harris, J.R., M.L. Cohen and E.C. Lippy. 1983. Water-related disease outbreaks in the United States, 1981. J. Infect. Dis. 148: 759.

Hejkal, T.W., B. Keswick, R.L. LaBelle, et al. 1982. Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious hepatitis. J. Am. Water Works Assoc. 74: 318.

Hendricks, C.W. 1978. Exceptions to the coliform and the fecal coliform tests. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Herrmann, J.E., K.D. Kostenbader, Jr. and D.O. Cliver. 1974. Persistence of enteroviruses in lake water. Appl. Microbiol. 28: 895.

Hill, W.F., Jr., E.W. Akin, W.H. Benton and T.G. Metcalf. 1972. Virus in water. II. Evaluation of membrane cartridge filters for recovering multiplicities of poliovirus in water. Appl. Microbiol. 23: 880.

Hoehn, R.C., C.W. Randall, E.A. Bell, Jr. and P.T.B. Shaffer. 1977. Trihalomethanes and viruses in a water supply. J. Environ. Eng. Div., ASCE. 103: 803.

Hoff, J.C. 1978. The relationship of turbidity to disinfection of potable water. In: Evaluation of the Microbiology Standards for Drinking Water, C.H. Hendricks, Ed. U.S. EPA, Cincinnati, OH.

Hoff, J.C. and R.C. Becker. 1969. The accumulation and elimination of crude and clarified poliovirus suspensions by shellfish. Am. J. Epidemiol. 90: 53.

Holmes, I.H. 1982. Basic rotavirus virology in humans. In: Virus Infections of the Gastrointestinal Tract, D.A.J. Tyrell and A.Z. Kapikian, Ed. Marcel-Dekker, Inc., New York.

Holzel, H., D.W. Cubitt, D.A. McSwiggan, P.J. Sanderson and J. Church. 1980. An outbreak of rotavirus infection among adults in a cardiology ward. J. Infect. 2: 33.

Howe, H.A. and J.L. Wilson. 1959. Poliomyelitis. In: Viral and Rickettsial Infections of Man, 3rd ed., T.M. Rivers and F.L. Horsfall, Jr., Ed. J.B. Lippincott Co., Philadelphia, PA.

IAWPRC Study Group on Water Virology. 1983. The health significance of viruses in water. Water Research. 17: 121.

Ignazzito, G., L. Volterra, F.A. Aulicino and A.M. D'Angelo. 1980. Coliphages as indicators in a treatment plant. Water, Air, Soil Pollut. 13: 391.

Jakubowski, W., J.C. Hoff, N.C. Anthony and W.F. Hill, Jr. 1974. Epoxy-fiberglass adsorbent for concentrating viruses from large volumes of potable water. Appl. Microbiol. 28: 501.

Jakubowski, W., W.F. Hill and N.A. Clarke. 1975. Comparative study of four microporous filters for concentrating viruses from drinking water. Appl. Microbiol. 28: 501.

Kapikian, A.Z., H.W. Kim, R.G. Wyatt, et al. 1976. Human reovirus-like agent as the major pathogen associated with winter gastroenteritis in hospitalized infants and young children. New Engl. J. Med. 294: 965.

Kapikian, A.Z., H.B. Greenberg, W.L. Cline, et al. 1978. Prevalence of antibody to the Norwalk agent by a newly developed immune adherence hemagglutination assay. J. Med. Virol. 2: 281.

Kapikian, A.Z., H.B. Greenberg, R.G. Wyatt, A.R. Kalica and R.M. Chanock. 1982. The Norwalk group of viruses - Agents associated with epidemic viral gastroenteritis. In: Virus Infections of the Gastrointestinal Tract, D.A.J. Tyrrell and A.Z. Kapikian, Ed. Marcel Dekker, Inc., New York.

Kaplan, J.E., G.W. Gary, R.C. Baron, et al. 1982. Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann. Int. Med. 96: 756.

Katz, M. and S.A. Plotkin. 1967. Minimal infective dose of attenuated poliovirus for man. Am. J. Pub. Health. 57: 1837.

Katz, M. and W.C. Price. 1967. Infection by viruses. Am. J. Pub. Health. 57: 1837.

Katzenelson, E., B. Fattal and T. Hostovesky. 1976. Organic flocculation: An efficient second step concentration method for the detection of viruses in tapwater. Appl. Environ. Microbiol. 32: 638.

Keeley, J.W. and R.S. Kerr. 1977. Meeting the ground water contamination problem. Water Sewage Works. 124: 88.

Kenner, B.A. 1978. Fecal streptococcal indicators. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Khuroo, M.S. 1980. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am. J. Med. 68: 818.

Kilbourne, E.D., C.B. Wilson and D. Perrier. 1956. The induction of gross myocardial lesions by a coxsackie (Pleurodynia) virus and cortisone. J. Clin. Invest. 35: 362.

Konno, T., H. Suzuki, N. Ishida, R. Chiba, R. Mochizuki and A. Tsunoda. 1982. Astrovirus-associated epidemic of gastroenteritis in Japan. J. Med. Virol. 9: 11.

Koopman, J.S., E.A. Eckert, H.B. Greenberg, B.C. Strohm, R.E. Isaacson and A.S. Monto. 1982. Norwalk virus enteric illness acquired by swimming exposure. Am. J. Epidemiol. 115: 173.

Koprowski, H. 1956. Immunization against poliomyelitis with living attenuated virus. Am. J. Trop. Med. Hyg. 5: 440.

Koprowski, H., T.W. Norton and W. McDermott. 1947. Isolation of poliomyelitis virus from human serum by direct inoculation into a laboratory mouse. Pub. Health Rep. 62: 1467.

Koprowski, H., T.W. Norton, G.A. Jerris, et al. 1956. Clinical investigations of attenuated strains of poliomyelitis virus: Use as a method of immunization of children with living virus. J. Am. Med. Assoc. 160: 954.

Kott, Y., N. Roze, S. Sperber and N. Betzer. 1974. Bacteriophages as viral pollution indicators. Water Res. 8: 165.

Kuehne, R.W., W.D. Sawyer and W.S. Gochenour, Jr. 1962. Infection with aerosolized attenuated Venezuelen equine encephalomyelitis virus. Am. J. Hyg. 75: 347.

Kurtz, J. and T. Lee. 1978. Astrovirus gastroenteritis age distribution of antibody. Med. Microbiol. Immunol. 166: 227.

LaBelle, R.L. and C.P. Gerba. 1980. Influence of estuarine sediment on virus survival under field conditions. Appl. Environ. Microbiol. 39: 749.

LaBelle, R.L., C.P. Gerba, S.M. Goyal, J.L. Melnick, I. Ceck and G. Bogden. 1980. Relationship between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. Appl. Environ. Microbiol. 39: 588.

Lee, T.W. and J.B. Kurtz. 1981. Serial propagation of astrovirus in tissue culture with the aid of trypsin. J. Gen. Virol. 57: 421.

Lemon, S.M., J.W. Le Duc, L. Binn, A. Escajadillo and K.G. Ishak. 1982. Transmission of hepatitis A virus among recently captured owl monkeys. J. Med. Virol. 10: 25.

Lennette, E.H. 1976. Epidemiology. In: Viruses in Water, G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, Ed. Am. Pub. Health Assoc., Washington, DC.

Lennette, E.H. and N.J. Schmidt, Ed. 1979. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. American Public Health Association, Inc., Washington, DC.

Levin, M.A. 1977. Bifidobacteria as water quality indicators. In: Bacterial Indicators/Health Hazards Associated With Water, A.W. Hoadley and B.J. Dutka, Ed. ASTM Special Tech. Pub. No. 635, American Society for Testing Materials, Philadelphia, PA.

Lippy, E.C. and S.C. Waltrip. 1984. Waterborne disease outbreaks: 1946-1980. A thirty-five year perspective. J. Am. Water Works Assoc. (In press)

Long, W.N. and F.A. Bell. 1972. Health factors and reused waters. J. Am. Water Works Assoc. 64: 220.

Marzouk, Y., S.M. Goyal and C.P. Gerba. 1979. Prevalence of enteroviruses in ground water of Israel. Groundwater. 17: 487.

Marzouk, Y., S.M. Goyal and C.P. Gerba. 1980. Relationship of viruses and indicator bacteria in water and wastewater of Israel. Water Res. 14: 1585.

Matthews, R.E.F. 1979. The classification and nomenclature of viruses. Intervirology. 11: 133.

Matthews, R.E.F. 1982. Classification and nomenclature of viruses. 4th Report of the International Committee on Taxonomy of Viruses. Intervirology. 17(1-3): 4.

McDaniels, A. and R.H. Bordner. 1983. Effects of holding time and temperature on coliform numbers in drinking water. J. Am. Water Works Assoc. 75: 458.

McNaughton, M.R. and H.A. Davies. 1981. Human enteric coronaviruses. Arch. Virol. 70: 301.

Melnick, J.L. 1971. Detection of virus spread by the water route. In: Viruses and Water Quality: Occurrence and Control, V. Snoeyink, Ed. Proc. 13th Water Quality Conf., University of Illinois, Urbana, IL.

Melnick, J.L. 1976. Viruses in water. In: Viruses in Water, G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, Ed. Am. Pub. Health Assoc., Washington, DC.

Melnick, J.L. 1978. Are conventional methods of epidemiology appropriate for risk assessment of virus contamination of water? *In*: Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, B.P. Sagik and C.A. Sorber, Ed. The Center for Applied Research and Technology, The University of Texas at San Antonio, TX.

Melnick, J.L. and C.P. Gerba. 1982. Viruses in surface and drinking waters. Environ. Internat. 7: 3.

Melnick, J.L., R. Safferman, V.C. Rao, et al. 1984. Round robin investigation of methods for the recovery of poliovirus from drinking water. Appl. Environ. Microbiol. 47: 144.

Metcalf, T.G. 1961. Use of membrane filters to facilitate the recovery of virus from aqueous suspensions. Appl. Microbiol. 9: 376.

Metcalf, T.G., B. Mullen, D. Eckerson, E. Moulton and E.P. Larkin. 1979. Bioaccumulation and depuration of enteroviruses by the soft-shelled clam, *Mya arenaria*. Appl. Environ. Microbiol. 38: 275.

Middleton, P.J., M. Szymanski, G.D. Abbott, R. Bortolussi and J.R. Hamilton. 1974. Orbivirus acute gastroenteritis of infancy. Lancet. 1: 1241.

Miller, W.S., P. Demchak, C.R. Rosenberger, J.W. Dominik and J.L. Bradshaw. 1963. Stability and infectivity of airborne yellow fever and Rift Valley fever viruses. Am. J. Hyg. 77: 114.

Minor, T.E., C.I. Allen, A.H. Tsiatis, D.B. Nelson and D.J. D'Alessio. 1981. Human infective dose determination for oral poliovirus type 1 vaccine in infants. J. Clin. Microbiol. 13: 38.

Moore, B.E., B.P. Sagik and C.A. Sorber. 1978. Land application of sludges: Minimizing the impact of viruses on water resources. In: Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, B.P. Sagik and C.A. Sorber, Ed. Center for Applied Research and Technology, The University of Texas at San Antonio, TX.

Moore, B.E., B.P. Sagik and C.A. Sorber. 1981. Viral transport to groundwater at a wastewater land application site. J. Water Pollut. Control Fed. 53: 1492.

Morris, R. 1981. Viruses in Water -- Are We Too Complacent? Paper presented at Meeting of IWES, Birmingham, January 9, 1981.

Morris, R. and W.M. Waite. 1981. Indicators, viruses and water quality. In: Viruses and Wastewater Treatment, M. Goddard and M. Butler, Ed. Pergamon Press, New York.

Mosley, J.W. 1967. Transmission of viral diseases by drinking water. In: Transmission of Viruses by the Water Route, G. Berg, Ed. John Wiley and Sons, New York.

Murphy, A.M., M.B. Albrey and E.B. Crieve. 1977. Rotavirus infections of neonates. Lancet. 2: 1149.

Nakata, S., S. Chiba, M. Terashima, Y. Sakuma, R. Kogasaka and T. Nakao. 1983. Microtiter solid-phase radioimmunoassay for detection of human calicivirus in stools. J. Clin. Microbiol. 17: 198.

Neefe, L.R. and J. Stokes, Jr. 1945. An epidemic of infectious hepatitis apparently due to a waterborne agent. J. Am. Med. Assoc. 128: 1063.

Nestor, I. and L. Costin. 1976. Presence of certain enteroviruses (coxsackie) in sewage effluents and river waters of Romania. J. Hyg. Epidemiol. Microbiol. Immunol. 20: 137.

Nestor, I., L. Costin, D. Sovrea and N. Ionescu. 1978. Investigations on the presence of enteroviruses in drinking water. Rev. Roum. Med. 29: 203.

Nicolas, J.C., J. Cohen, B. Fortier, M.H. Lourenco and F. Bricout. 1983. Isolation of a human pararotavirus. Virology. 124: 181.

Nupen, E.M. and G.J. Stander. 1973. The virus problem in the Windhoek waste water reclamation project. In: Advances in Water Pollution Research, S.H. Jenkins, Ed. Pergamon Press, New York.

Okuno, Y. 1962. Vaccination with egg passage measles virus by inhalation. Am. J. Dis. Children. 103: 381.

Pappenheimer, A.M., L.J. Kunz and S. Richardson. 1951. Passage of coxsackievirus (Connecticut-5 strain) in adult mice with production of pancreatic disease. J. Exp. Med. 94: 45.

Parrino, T.A., D.S. Schreiber, J.S. Trier, A.Z. Kapikian and N.R. Blacklow. 1977. Clinical immunity in acute gastroenteritis caused by Norwalk agent. New Engl. J. Med. 297: 86.

Pavri, K., M.A. Sreenivasan, A. Sehgal and S.R. Prasad. 1982. Waterborne epidemics of non-A non-B viral hepatitis in India. In: Viral Diseases in South-East Asia and the Western Pacific, J.S. MacKenzie, Ed. Academic Press, New York.

Payment, P. 1981. Isolation of viruses from drinking water at the Pont-Viau water treatment plant. Canad. J. Microbiol. 27: 417.

Payment, P. 1984. Elimination of viruses during treatment of surface waters for the preparation of drinking water. Newsletter on Water Virology, E.W. Akin and W.O.K. Grabow, Ed. IAWPRC, No. 4, January, 1984.

Petrilli, F.L., P. Crovari, S. DeFlora and A. Vannucci. 1974. The virological monitoring of water. I. Drinking water. Boll. Ist. Sieroter, Milan. 53: 434.

Plotkin, S.A. and M. Katz. 1967. Minimal infective doses of viruses for man by the oral route. In: Transmission of Viruses by the Water Route, G. Berg, Ed. John Wiley and Sons, New York.

Primrose, S.B., N.D. Seeley and K. Logan. 1981. The recovery of viruses from water: Methods and applications. In: Viruses and Wastewater Treatment, M. Goddard and M. Butler, Ed. Pergamon Press, New York.

Provost, P.J., W.J. McAleer and M.R. Hilleman. 1982. In vitro cultivation of hepatitis A virus. In: Viral Hepatitis, W. Szmuness, J.H. Alter and J.E. Maynard, Ed. The Franklin Institute Press, Philadelphia, PA.

Purcell, R.H. 1982. The hepatitis viruses: An overview and historical perspective. In: Viral Hepatitis, W. Szmuness, H.J. Alter and J.E. Maynard, Ed. The Franklin Institute Press, Philadelphia, PA.

Quality Criteria for Water. 1977. U.S. EPA, Office of Water and Hazardous Materials.

Rabyshko, E.V. 1974. Certain problems of the circulation of enteroviruses in the environmental objects. Gig. Sanit. 39: 105.

Rao, V.C., S.B. Lakhe and S.V. Wahgmari. 1978. Developments in environmental virolgy in India. IAWPRC Tech. Am. Rpt. 5: 1.

Report to Congress. 1978. Human Viruses in the Aquatic Environment: A Status Report With Emphasis on the EPA Research Program. EPA 570/9-78-006.

Riepenhoff-Talty, M., L.J. Saif, H.J. Barrett, H. Suzuki and P.L. Ogra. 1983. Potential spectrum of etiological agents of viral enteritis in hospitalized infants. J. Clin. Microbiol. 17: 352.

Robbins, S.L. and R.S. Cotran. 1979. Pathologic Basic of Disease. W.B. Saunders Co., Philadelphia, PA. p. 1037.

Ryder, R.W., C.A. Oquist, H. Greenberg, et al. 1981. Travellers' diarrhea in Panamanian tourists in Mexico. J. Infect. Dis. 144: 442.

Sabin, A.B. 1955. Personal communication.

Sabin, A.B. 1956. Pathogenesis of poliomyelitis. Science. 123: 1151.

Sakuma, Y., S. Chiba, R. Kogasaka, et al. 1981. Prevalence of antibody to human calicivirus in general population of Northern Japan. J. Med. Virol. 7: 221.

Saulsbury, F.T., J.A. Winkelstein and R.H. Yolken. 1980. Chronic rotavirus infection in immunodeficiency. J. Pediatrics. 97: 61.

Scarpino, P.V. 1978. Bacteriophage indicators. In: Indicators of Viruses in Water and Food, G. Berg, Ed. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Scarpino, P.V. 1984. Personal communication.

Schaffer, F.L. 1979. Calicivirus. Comp. Virol. 14: 249.

Schiff, G.M., G. Stefanovic, E. Young and J. Pennekamp. 1983. Determination of Minimal Infectious Dose of an Enterovirus in Drinking Water. Final Report to the U.S. EPA, Cincinnati, OH.

Schmidt, N.J., H.H. Ho and E.H. Lennette. 1975. Propagation and isolation of group A coxsackieviruses in RD cells. J. Clin. Microbiol. 2: 183.

Schmidt, N.J., H.H. Ho, J.L. Riggs and E.H. Lennette. 1978. Comparative sensitivities of various cell culture systems for isolation of viruses from wastewater and fecal samples. Appl. Environ. Microbiol. 36: 480.

Schreiber, D.S., N.R. Blacklow and J.S. Trier. 1973. The mucosal lesion in acute infectious nonbacterial gastroenteritis. New Engl. J. Med. 288: 1318.

Sekla, L., W. Stackiw, C. Kay and L. Van Backenhout. 1980. Enteric viruses in renovated water in Manitoba. Can. J. Microbiol. 26: 518.

Sheridan, J.F., L. Aurellian, L. Barbour, M. Santosham, R.B. Sack and R.W. Ryder. 1981. Travellers' diarrhea associated with rotavirus infection: Analysis of virus-specific immunoglobulin classes. Infect. Immunol. 31: 419.

Shuval, H.I. 1976. Water needs and usage. In: Viruses in Water, G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, Ed. Am. Pub. Health Assoc., Washington, DC.

Shuval, H.I. and E. Katzenelson. 1972. The detection of enteric viruses in the water environment. In: Water Pollution Microbiology, R. Mitchell, Ed. Interscience Publishers, New York.

Shuval, H.I., A. Tompson, B. Fattal, S. Cymbalista and Y. Wiener. 1971. Natural Virus Inactivation Processes in Seawater. In: Proc. Natl. Specialty Conf. Disinfect. Am. Soc. Civil Eng., New York.

Simkova, A. and J. Cervenka. 1981. Coliphages as ecological indicators of enteroviruses in various water systems. Bull. WHO. 59: 611.

Slade, J.S. and B.J. Ford. 1983. Discharge to the environment of viruses in wastewater, sludges, and aerosols. In: Viral Pollution of the Environment, G. Berg, Ed. CRC Press Inc., Boca Raton, FL.

Smith, A.W., D.E. Skilling, A.H. Dardiri and A.B. Latham. 1980. Calicivirus pathogenic for swine: A new serotype isolated from opaleye *Girella nigricans*, an ocean fish. Science. 209: 940.

Smith, E.M. and C.P. Gerba. 1981. Survival and Detection of Rotaviruses in the Environment. Proc. Third Intern. Symp. Neonatal Diarrhea, S.D. Acres, A.J. Forman and H. Fast, Ed. Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan.

Smith, E.M. and C.P. Gerba. 1982. Laboratory methods for the growth and detection of animal viruses. In: Methods in Environmental Virology, C.P. Gerba and S.M. Goyal, Ed. Marcel Dekker, Inc., New York.

Smith, E.M., C.P. Gerba and J.L. Melnick. 1978. Role of sediments in the persistence of enteroviruses in the estuarine environment. Appl. Environ. Microbiol. 35: 685.

Sobsey, M.D. and J.S. Glass. 1980. Poliovirus concentration from tapwater with electropositive adsorbent filters. Appl. Environ. Microbiol. 4: 261.

Sobsey, M.D., S.E. Oglesbee and D.A. Wait. 1984. Detection of hepatitis A virus in drinking water. (In press)

Sorber, C.A. 1983. Removal of viruses from wastewater and effluent by treatment processes. In: Viral Pollution of the Environment, G. Berg, Ed. CRC Press Inc., Boca Raton, FL.

Sproul, O.J. 1976. Removal of viruses by treatment processes. In: Viruses in Water, G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, Ed. Am. Pub. Health Assoc., Washington, DC.

Sreenivasan, M.A., V.A. Arankalle, A. Sehgal and K.M. Pavri. 1984. Non-A, non-B epidemic hepatitis: Visualization of virus-like particles in the stool by immune electron microscopy. J. Gen. Virol. 65: 1005.

State of Arizona. 1981. Water Quality Standards for Surface Waters. 89-21-100.

Steinmann, J. and G. Havemeister. 1982. Elimination of bacteria and viruses by flocculation in treated sewage. Zbl. Bakt., I. Abt. Orig. B176: 546.

Stetler, R.E. 1984. Coliphages as indicators of enteric viruses. (In press)

Stetler, R.E., R.L. Ward and S.C. Waltrip. 1984. Enteric virus and indicator bacteria levels in a water treatment system modified to reduce trihalomethane production. Appl. Environ. Microbiol. 47: 319.

Suptel, E.A. 1963. Pathogenesis of experimental coxsackievirus infection. Acta Virol. 7: 61.

Takiff, H.E., S.E. Strauss and C.F. Garon. 1981. Propagation and *in vitro* studies of previously non-cultivatable enteral adenoviruses in 293 cells. Lancet. iii: 832.

Taniguchi, K., S. Urasawa and T. Urasawa. 1979. Virus-like particles 35-40 nm, associated with an institutional outbreak of acute gastroenteritis in adults. J. Clin. Microbiol. 10: 730.

Taniguchi, K., S. Urasawa and T. Urasawa. 1981. Further studies of 35-40 nm virus-like particles associated with outbreaks of acute gastroenteritis. J. Med. Microbiol. 14: 107.

Totterdell, B.M., I.L. Chrystie and J.E. Banatvala. 1976. Rotavirus infections in a maternity unit. Arch. Dis. Child. 51: 924.

Tyler, J.M. 1983. Viruses in fresh and saline waters. *In*: Viruses and Disinfection of Water and Wastewater, M. Butler, B. Utler, A.R. Medlen and R. Morris, Ed. University of Surrey Press Unit, Surrey, UK.

Uhnoo, I., G. Waddell, L. Svensson and M. Johansson. 1983. Two new serotypes of enteric adenovirus causing infantile diarrhea. Develop. Biol. Stand. 53: 311.

Vaughn, J.M. and T.G. Metcalf. 1975. Coliphages as indicators of enteric viruses in shellfish and shellfish raising estuarine waters. Water Res. 9: 613.

Vaughn, J.M., E.F. Landry, L.J. Baranosky, C.A. Beckwith, M.C. Dahl and N.C. Delias. 1978. Survey of human virus occurrence in wastewater-recharged groundwater on Long Island. Appl. Environ. Microbiol. 36: 47.

Vesikari, T., M. Maki, H.K. Sarkkinen, P.P. Arstila and P.E. Halonen. 1981. Rotavirus, adenovirus, and non-viral enteropathogens in diarrhea. Arch. Dis. Child. 56: 264.

Viswanathan, R. 1957. Certain epidemiological features of infectious hepatitis during Delhi epidemic 1955-1956. In: Hepatitis Frontiers, F.W. Hartman et al., Ed. Henry Ford Hopital, International Symposium, No. 6, Little, Brown, Boston, MA.

Wallis, C. and J.L. Melnick. 1967. Concentration of viruses from sewage by adsorption on millipore membranes. Bull. WHO. 36: 219.

Wallis, C., M. Henderson and J.L. Melnick. 1972. Enterovirus concentration on cellulose membranes. Appl. Microbiol. 23: 46.

Ward, R.R. and E.W. Akin. 1984. Minimum infectious dose of animal viruses. CRC Crit. Rev. Environ. Control. 14(4): 297-310.

Ward, R.L. and C.S. Ashley. 1980. Effects of wastewater sludge and its detergents on the stability of rotavirus. Appl. Environ. Microbiol. 39: 1154.

Wellings, F.M. 1982. Viruses in groundwaters. In: Significance of Viruses in the Environment, G. Berg, Ed. Environ. Internat. 7: 9.

Wellings, F.M., A.L. Lewis and C.W. Mountain. 1973. Is a virus standard necessary? J. Am. Water Works Assoc., AWWA Water Quality Technology Conference, Cincinnati, OH, December 3 and 4.

Wellings, F.M., A.L. Lewis and C.W. Mountain. 1974. Virus survival following wastewater spray irrigation of sandy soils. In: Virus Survival in Water and Wastewater Systems, J.M. Malina and B.P. Sagik, Ed. Center for Research in Water Resources, University of Texas at Austin, Austin, TX.

Wellings, F.M., C.W. Mountain and A.L. Lewis. 1975. Virus in groundwater. Proc. 2nd Natl. Conf. on Individual Onsite Wastewater Systems, Natl. Sanit. Fnd., Ann Arbor, MI.

Wellings, F.M., A.L. Lewis and C.W. Mountain. 1977. Assessment of health risks associated with land disposal of municipal effluents and sludge. In: Proc. Conf. on Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, B.P. Sagik and C.A. Sorber, Ed. Center for Applied Research and Technology, University of Texas at San Antonio, TX.

Wenman, W.M., D. Hinde, S. Feltham and M. Furwith. 1979. Rotavirus infection in adults: Results of a prospective family study. New Engl. J. Med. 301: 303.

Westwood, J.C.N. and S.A. Sattar. 1976. The minimal infective dose. In: Viruses in Water, G. Berg, H.R. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, Ed. Am. Pub. Health Assoc., Washington, DC.

WHO (World Health Organization). n.d. Guidelines for Drinking Water Quality, Volume I, Recommendations, WHO, Geneva. (To be published)

WHO (World Health Organization). 1972. International Standards for Drinking Water, 3rd ed. WHO, Geneva.

WHO (World Health Organization). 1973. Reuse of effluents: Methods of Wastewater Treatment and Health Safeguards. Technical Report Series No. 517. Geneva.

WHO Scientific Group. 1979. Human Viruses in Water. Technical Support Series 639, World Health Organization, Geneva.

Wong, D.C., R.H. Purcell, M.A. Sreenivasan, S.R. Prasad and K.M. Pavri. 1980. Epidemic and endemic hepatitis in India: Evidence for non-A, non-B hepatitis virus aetiology. Lancet. II: 876.

Wyatt, R.G., H.D. James, A.L. Pittman, et al. 1983. Direct isolation in cell culture of human rotaviruses and their characterization into four serotypes. J. Clin. Microbiol. 18: 310.

Wyatt, R.G., R. Dolin, N.R. Blacklow, et al. 1984. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. J. Infect. Dis. 129: 709.

Yolken, R.H., F. Laurence, F. Leister, H.E. Takiff and S.E. Strauss. 1982. Gastroenteritis associated with enteric type adenovirus in hospitalized infants. J. Pediatrics. 101: 21.